Electronic supplementary materials *Mendeleev Commun*., 2023, **33**, 627–630

Antimicrobial agents based on new lipophilic alkyl (*N*-alkyl-*N*,*N*-dioctylammoniomethyl)phosphonates

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I. Experimental

All reagents used in the synthesis were supplied by Acros Organics, Alfa Aesar or were of the highest quality available. Commercially available solvents were purified by standard procedures. Amino phosphonates were obtained by the Kabachnik-Fields reaction according to the standard method [S1]. Methyl iodide, ethyl iodide and benzyl chloride was redistilled, their physical parameters were compared with literature data. *p*-Toluenesulfonic acid 97.5% chemical grade were used.

NMR spectra were recorded at room temperature in $CDCl_3$ solution on a Bruker Avance III 400 spectrometer with an operating frequency of 162 MHz for ^{31}P spectra, an operating frequency of 400 MHz for ^{1}H spectra and an operating frequency of 100.6 MHz for ^{13}C spectra in a solution of $CDCl_3$. The Fourier Transformed InfraRed (FT-IR) spectrum of the sample was recorded using Perkin Elmer UATR Two FT-IR Spectrometer (Spectrum Two) with an ATR (attenuated total reflectance) diamond crystal. Spectra were baseline corrected and normalized. CHN analysis was performed on a vario EL cube elemental analyzer (Elementar, Germany).

General procedure for the synthesis of potassium alkyl (dioctylaminomethyl)phosphonates 1a-c

A mixture of 2 mM of dialkyl (dioctylaminomethyl)phosphonate and 2mM of KOH (20 wt % excess) in 10 ml of 1,4-dioxane was heated under reflux for 10 h. After removal of the solvents the resulting salt dried *in vacuo*.

Potassium butyl (dioctylaminomethyl)phosphonates 1a

Yield 80%. FTIR spectrum, cm^{-1}: 1063 (P-O-C), 1198 (P=O). ^{31}P NMR (1,4- dioxane): δ_P 19.5 ppm

Potassium isobutyl (dioctylaminomethyl)phosphonates 1b

Yield 80%. FTIR spectrum, cm^{-1}: 1040 (P-O-C), 1194 (P=O). ^{31}P NMR (1,4- dioxane): δ_P 19.6 ppm

Potassium hexyl (dioctylaminomethyl)phosphonates 1c

Yield 78%. FTIR spectrum, cm^{-1}: 1060 (P-O-C), 1196 (P=O). ^{31}P NMR (1,4- dioxane): δ_P 19.5 ppm

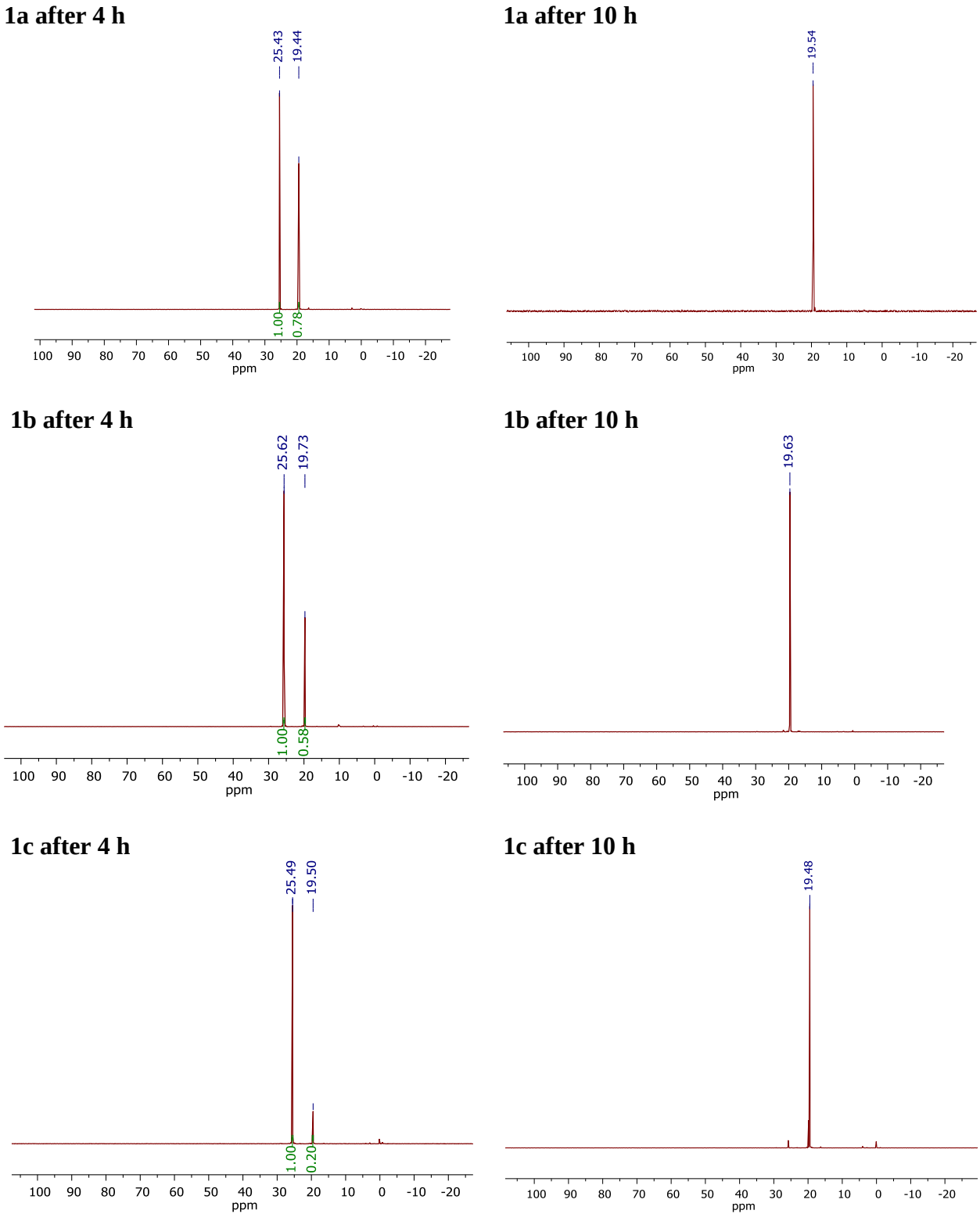
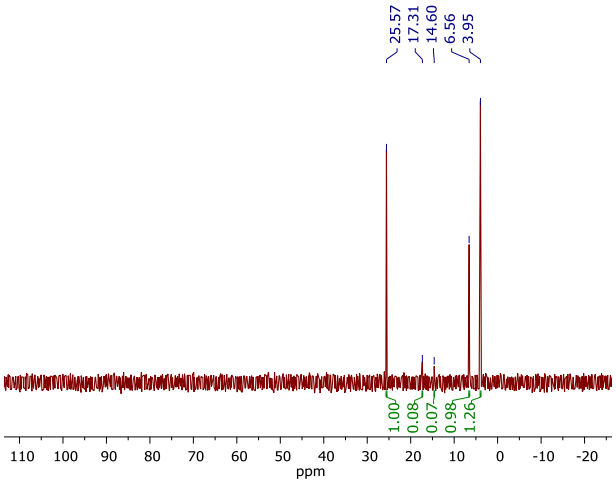
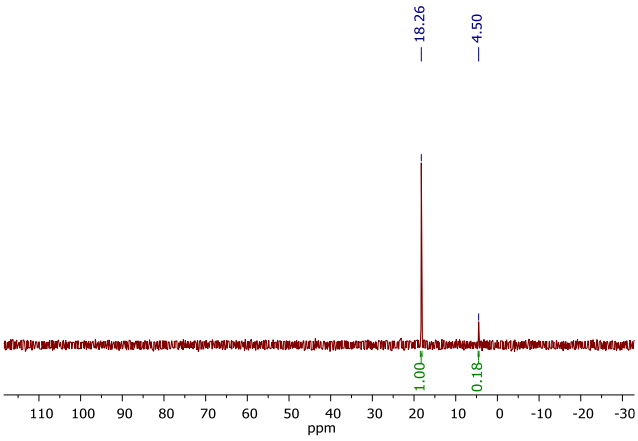


Figure S1 $^{31}P\{^{1}H\}$ NMR spectra of the reaction mixtures of the compounds **1a-c** respectively

2a in DMF after 3 h



2a in propan-2-ol after 1 h



2a in propan-2-ol after 3 h

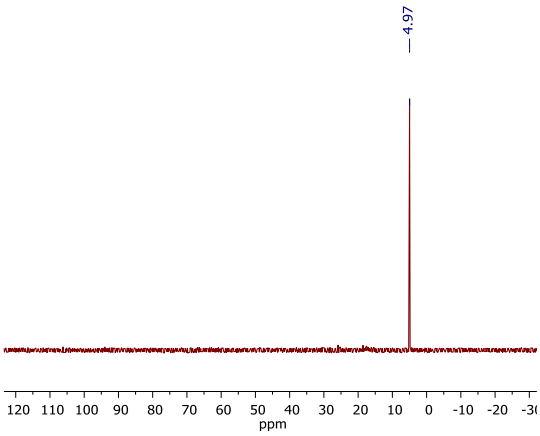


Figure S2 $^{31}P\{^{1}H\}$ NMR spectra of the reaction mixtures of the compound **2a** respectively

Synthesis of alkyl (*N*-alkyl-*N*,*N*-dioctylammoniomethyl)phosphonates 2a-i

A mixture of 3.5 mM potassium alkyl (dioctylaminomethyl)phosphonates, 3.5 mM alkyl iodide or benzyl chloride was stirred ($t_{bath} \approx 60°C$) under reflux in 10 ml of propan-2-ol for 3 h. Propan-2-ol was evaporated under vacuum, the resulting product is dissolved in chloroform, precipitate of potassium halide is separated by centrifugation. Then the solvent is removed, the residue is decanted with light petroleum ether and acetonitrile, then dried in a vacuum desiccator.

The syntheses and spectral characteristics of compounds **2g** and **2i** were described in [S2].

Butyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)phosphonate 2a

Yield 83 %, thick oil. FTIR spectrum, cm^{-1}: 1081, 1066, 1043, 1029 (P-O-C), 1220 (P=O). ^1H NMR ($CDCl_3$), δ_H, ppm. : 0.85 t. (6H, $[C\underline{H}_3]_2$, $^3J_{HH}$ 6.45 Hz), 0.89 t. (3H, $C\underline{H}_3$ $^3J_{HH}$ 7.28 Hz), 1.15-1.95 m. (28H $CH_3(C\underline{H}_2)_6$, $CH_3(C\underline{H}_2)_2CH_2O$), 3.33 s. (3H, $NC\underline{H}_3$), 3.40-3.62 m. (6H, $PC\underline{H}_2N$, $NC\underline{H}_2CH_2$) 3.85-4.02 m. (2H, $C\underline{H}_2OP$). $^{13}C\{^1H\}$ NMR ($CDCl_3$), δ_C, ppm.: 13.76 s., 13.98 s. 18.96 s., 22.49 s., 22.60 s., 26.19 s., 28.99 s., 29.08 s., 31.55 s., ($\underline{C}H_3(\underline{C}H_2)_6$, $\underline{C}H_3\underline{C}H_2CH_2CH_2O$), 33.06 d. ($POC\underline{C}H_2$, $^3J_{CP}$ 6.57 Hz), 50.35 s. ($\underline{C}H_3N$), 58.74 d. ($N\underline{C}H_2P$, $^1J_{CP}$ 126.91 Hz), 63.26 d. ($N\underline{C}H_2$, $^3J_{CP}$ 4.58 Hz), 64.41 d. ($\underline{C}H_2OP$, $^2J_{CP}$ 5.98 Hz). $^{31}P\{^1H\}$ NMR (propan-2-ol): δ_P 4.6 ppm.

Found (%): C, 64.86; H, 11.78; N, 3.38; O, 11.51. Calc. for $C_{22}H_{48}NO_3P$ (%): C, 65.15 ; H, 11.93; N, 3.45; O, 11.93.

Butyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate 2b

Yield 75 %, thick oil.

FTIR spectrum, cm^{-1}: 1081, 1063, 1044, 1029 (P-O-C), 1216 (P=O)**.** ^1H NMR ($CDCl_3$), δ_H, ppm.: 0.88 t. (6H, $[C\underline{H}_3]_2$, $^3J_{HH}$ 6.73 Hz), 0.92 t. (3H, $C\underline{H}_3$ $^3J_{HH}$ 7.4 Hz), 1.14-1.91 m. (31H $CH_3(C\underline{H}_2)_6$, $CH_3(C\underline{H}_2)_2CH_2O$, $C\underline{H}_3CH_2N$), 3.75 d. (2H, $PC\underline{H}_2N$, $^2J_{PH}$ 13.28 Hz), 3.39-3.50 m. (4H, $NC\underline{H}_2CH_2$), 3.66 q. (2H, $NC\underline{H}_2CH_3$ $^1J_{HH}$ 6.76 Hz), 4.02-4.15 m. (2H, $C\underline{H}_2OP$). $^{13}C\{^1H\}$ NMR ($CDCl_3$), δ_C, ppm.: 8.10 s., 13.76 s., 13.97 s. 18.96 s., 22.17 s., 22.49 s., 26.28 s., 29.00 s., 29.06 s., 31.55 s., 31.55 s. ($\underline{C}H_3(\underline{C}H_2)_6$, $\underline{C}H_3\underline{C}H_2CH_2CH_2O$, $\underline{C}H_3CH_2N$), 33.14 d. ($POC\underline{C}H_2$, $^3J_{CP}$ 6.37 Hz), 55.35 d. ($N\underline{C}H_2P$, $^1J_{CP}$ 130.00 Hz), 55.59 s. ($N\underline{C}H_2CH_3$), 59.83 s. ($N\underline{C}H_2CH_2$), 64.21 s. ($\underline{C}H_2OP$). $^{31}P\{^1H\}$ NMR (propan-2-ol): δ_P 4.9 ppm.

Found (%): C, 65.61; H, 11.89; N, 3.24; O, 11.08. Calc. for $C_{23}H_{50}NO_3P$ (%): C, 65.83; H, 12.01; N, 3.34; O, 11.44.

Butyl (*N*-benzyl-*N*,*N*-dioctylammoniomethyl)phosphonate 2c

Yield 81 %, thick oil.

FTIR spectrum, cm^{-1}: 1096, 1066, 1047, 1030 (P-O-C), 1236 (P=O)**.** ^{1}H NMR ($CDCl_3$), δ_H, ppm.: 0.86 t. (6H, $[C\underline{H}_3]_2$, $^{3}J_{HH}$ 7.69 Hz), 0.88 t. (3H, $C\underline{H}_3$ $^{3}J_{HH}$ 7.48 Hz), 1.20-1.61 m. (28H $CH_3(C\underline{H}_2)_6$, $CH_3(C\underline{H}_2)_2$) 3.17 d. (2H, $PC\underline{H}_2N$, $^{2}J_{PH}$ 12.29 Hz), 3.35 dt. (4H, $NC\underline{H}_2$, $^{2}J_{H1H2}$ 12.40, $^{3}J_{HH}$ 3.89 Hz), 3.90-4.00 m. (2H, $C\underline{H}_2OP$), 4.89 s. (2H, $C\underline{H}_2C_6H_5$), 7.42-7.66 m. (5H, $C_6\underline{H}_5$). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$), δ_C, ppm.: 13.77 s., 13.99 s., 18.99 s., 22.45 s. 22.49 s., 26.18 s., 28.98 s., 29.06 s., 31.54 s., ($\underline{C}H_3(\underline{C}H_2)_6$, $\underline{C}H_3\underline{C}H_2CH_2CH_2O$), 33.23 d. ($POC\underline{C}H_2$, $^{3}J_{CP}$ 6.28 Hz), 57.56 d. ($N\underline{C}H_2P$, $^{1}J_{CP}$ 126.91 Hz), 59.81 d. ($N\underline{C}H_2$, $^{3}J_{CP}$ 4.58 Hz), 62.99 s. ($N\underline{C}H_2Ph$), 64.14 d. ($\underline{C}H_2OP$, $^{2}J_{CP}$ 5.90 Hz), 127.26 s. 129.29 s. 130.67 s. 132.93 s. (C_6H_5). $^{31}P\{^{1}H\}$ NMR (propan-2-ol): δ_P 4.3 ppm.

Found (%): C, 69.46; H, 10.79; N, 2.81; O, 9.75. Calc. for $C_{28}H_{52}NO_3P$ (%): C, 69.82; H, 10.88; N, 2.91; O, 9.96.

Isobutyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)phosphonate 2d

Yield 86 %, thick oil.

FTIR spectrum, cm^{-1}: 1082, 1035 (P-O-C), 1231 (P=O)**.** ^{1}H NMR ($CDCl_3$), δ_H, ppm.: 0.85 t. (6H, $[C\underline{H}_3]_2$, $^{3}J_{HH}$ 6.60 Hz), 0.89 d. (6H, $(C\underline{H}_3)_2CH$, $^{3}J_{HH}$ 6.67 Hz), 1.22-1.97 m. (25H, $CH_3(C\underline{H}_2)_6$, $(CH_3)_2C\underline{H}CH_2$), 3.34 s. (3H, $C\underline{H}_3N$), 3.40-3.53 m. (6H, $PC\underline{H}_2N$, $NC\underline{H}_2CH_2$), 3.63-3.70 m. (2H, $C\underline{H}_2OP$). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$), δ_C, ppm.: 14.00 s., 19.09 s., 22,51 s., 22.59 s., 26.22 s., 29.09 s., 29.40 s., 29.46 s., 31.57 s., ($\underline{C}H_3(\underline{C}H_2)_6$, $[(\underline{C}H_3)_2\underline{C}HCH_2]$), 50.36 s. ($\underline{C}H_3N$), 58.98 d. ($N\underline{C}H_2P$, $^{1}J_{CP}$ 125.36 Hz), 63.07 s. ($N\underline{C}H_2$), 70.75 d. ($\underline{C}H_2OP$, $^{2}J_{CP}$ 6.31 Hz). $^{31}P\{^{1}H\}$ NMR (propan-2-ol): δ_P 5.2 ppm.

Found (%): C, 65.04; H, 11.73; N, 3.36; O, 11.76. Calc. for $C_{22}H_{48}NO_3P$ (%): C, 65.15; H, 11.93; N, 3.45; O, 11.93.

Isobutyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate 2e

Yield 83 %, thick oil.

FTIR spectrum, cm^{-1}: 1036, 1082 (P-O-C), 1226 (P=O)**.** ^{1}H NMR ($CDCl_3$), δ_H, ppm.: 0.82 t. (6H, $[C\underline{H}_3]_2$, $^{3}J_{HH}$ 6.67 Hz), 0.85 d. (6H, $[(C\underline{H}_3)_2CH]_2$, $^{3}J_{HH}$ 6.61 Hz), 0.87-1.84 m. (28H, $CH_3(C\underline{H}_2)_6$, $(CH_3)_2C\underline{H}CH_2$, $C\underline{H}_3CH_2N$), 3.29 d. (2H, $PC\underline{H}_2N$, $^{2}J_{PH}$ 12.4 Hz), 3.33-3,43 m. (4H, $NC\underline{H}_2CH_2$), 3.55-3.61 m. (2H, $NC\underline{H}_2$), 3.64-3.69 m. (2H, $C\underline{H}_2OP$). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$), δ_C, ppm.: 8.16 s., 13.90 s., 18.95s., 22,12 s., 22.41 s., 26.21 s., 28.92 s., 28.97 s., 31.47 s., ($\underline{C}H_3(\underline{C}H_2)_6$, $[(\underline{C}H_3)_2CHCH_2]$, $\underline{C}H_3CH_2N$), 29.37 d. ($POC\underline{C}H$, $^{3}J_{CP}$ 6.29 Hz), 55.15 d. ($N\underline{C}H_2P$, $^{1}J_{CP}$ 129.43 Hz), 55.67 s. ($N\underline{C}H_2CH_3$), 59.83 s. ($N\underline{C}H_2CH_2$), 70.67 d. ($\underline{C}H_2OP$, $^{2}J_{CP}$ 6.32 Hz). $^{31}P\{^{1}H\}$ NMR (propan-2-ol): δ_P 3.8 ppm.

Found (%): C, 65.79; H, 11.87; N, 3.27; O, 11.24. Calc. for $C_{23}H_{50}NO_3P$ (%): C, 65.83; H, 12.01; N, 3.34; O, 11.44.

Isobutyl (*N*-benzyl-*N,N*-dioctylammoniomethyl)phosphonate 2f

Yield 80 %, thick oil.

FTIR spectrum, cm^{-1}: 1040, 1082 (P-O-C), 1231(P=O)**.** ^{1}H NMR ($CDCl_3$), δ_H, ppm.: 0.89 t. (6H, [C<u>H</u>$_3$]$_2$, $^3J_{HH}$ 6.56 Hz), 0.93 d. (6H, [(C<u>H</u>$_3$)$_2$CH]$_2$, $^2J_{HH}$ 6.68 Hz), 1.15-2.21 m. (25H, CH_3(C<u>H</u>$_2$)$_6$, [$(CH_3)_2$C<u>H</u>CH_2]), 3.28 d. (2H, PC<u>H</u>$_2$N, $^2J_{PH}$ 12.24 Hz), 3.21 dt. (2H, NC'<u>H</u>$_2$, $^2J_{H1H2}$ 12.88, $^3J_{HH}$ 3.86 Hz), 3.38 dt. (2H, NC<u>H</u>$_2$, $^2J_{H1H2}$ 12.88, $^3J_{HH}$ 3.86 Hz), 3.76-3.80 m. (2H, C<u>H</u>$_2$OP), 4.90 s. (2H, C<u>H</u>$_2$C$_6$H$_5$), 7.30-7.67 m. (5H, C$_6$<u>H</u>$_5$). ^{13}C{^{1}H} NMR ($CDCl_3$), δ_C, ppm.: 14.02 s., 18.96 s., 22.52 s., 22.62 s, 26.24 s., 29.05 s., 29.36 s., 29.42 s., 31.56 s., (<u>C</u>H$_3$(<u>C</u>H$_2$)$_6$, [(<u>C</u>H$_3$)$_2$<u>C</u>H]), 55.01 d. (N<u>C</u>H$_2$P, $^1J_{CP}$ 125.36 Hz), 60.16 d. (N<u>C</u>H$_2$, $^3J_{CP}$ 3.41 Hz), 71.17 d. (<u>C</u>H$_2$OP, $^2J_{CP}$ 6.31 Hz), 63.61 s. (N<u>C</u>H$_2$Ph), 127.38 s. 129.29 s. 130.70 s. 132.94 s. (<u>C</u>$_6$H$_5$). ^{31}P{^{1}H} NMR (propan-2-ol): δ_P 3.6 ppm.

Found (%): C, 69.51; H, 10.53; N, 2.90; O, 9.86. Calc. for $C_{28}H_{52}NO_3P$ (%): C, 69.82; H, 10.88; N, 2.91; O, 9.96.

Hexyl (*N*-ethyl-*N,N*-dioctylammoniomethyl)phosphonate 2h

Yield 80 %, thick oil.

FTIR spectrum, cm^{-1}: 1022, 1061, 1084 (P-O-C), 1233(P=O^{1}H NMR ($CDCl_3$), δ_H, ppm.: 0.88 t. (9H, [C<u>H</u>$_3$]$_2$, C<u>H</u>$_3$ $^3J_{HH}$ 7.04 Hz), 1.25-1.75 m. (35H, CH_3(C<u>H</u>$_2$)$_6$, CH_3(C<u>H</u>$_2$)$_4$$CH_2O$, C<u>H</u>$_3$$CH_2N$), 3.40-3.68 m. (8H, PC<u>H</u>$_2$N, NC<u>H</u>$_2$$CH_3$), 3.98-4.03 m. (2H, C<u>H</u>$_2$OP). ^{13}C{^{1}H} NMR ($CDCl_3$), δ_C, ppm.: 8.18 s., 13.98 s., 22.21 s., 22.50 s., 22.55 s., 25.46 s., 26.30 s., 29.02 s., 29.08 s., 31.56 s. (<u>C</u>H$_3$(<u>C</u>H$_2$)$_6$, <u>C</u>H$_3$(<u>C</u>H$_2$)$_4$$CH_2O$, <u>C</u>H$_3$$CH_2N$), 31.10 d. (POC<u>C</u>H$_2$, $^3J_{CP}$ 6.44 Hz), 55.41 d. (N<u>C</u>H$_2$P, $^1J_{CP}$ 128.95 Hz), 55.62 s. (<u>C</u>H$_2$N), 59.83 d. (N<u>C</u>H$_2$ $^2J_{CP}$ 2.62 Hz), 64.53 d. (<u>C</u>H$_2$OP, $^2J_{CP}$ 4.53 Hz). ^{31}P{^{1}H} NMR (propan-2-ol): δ_P 4.4 ppm.

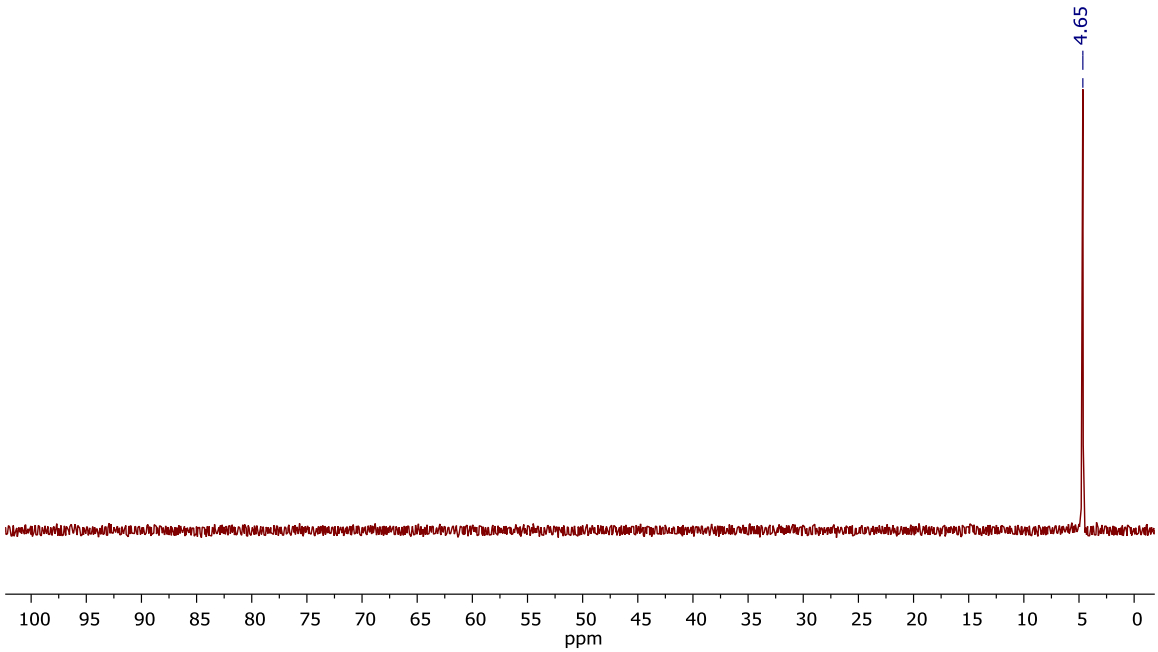
Found (%): C, 67.04; H, 11.94; N, 3.10; O, 10.50. Calc. for $C_{25}H_{54}NO_3P$ (%): C, 67.07; H, 12.16; N, 3.13; O, 10.72.

Antibacterial activity

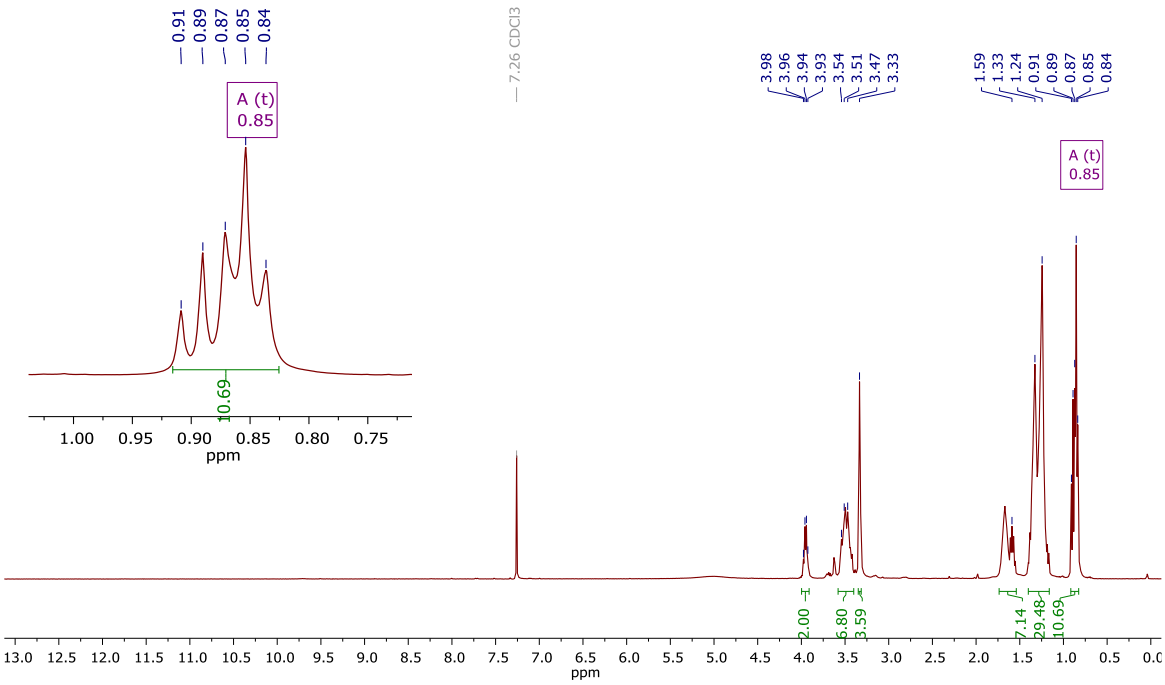
The antimicrobial activity of the synthesized compounds **2a-i** was determined *in vitro* using the agar disk-diffusion method using Mueller-Hilton agar medium against a variety of pathogenic microorganisms in DMSO or 1,2 - DCB: *Staphylococcus aureus* (ATCC 29213), *Bacillus cereus* (ATCC 11778), as well as with Gram-negative cultures: *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), Klebsiella pneumonia (ATTC 13883). Chlorhexidine (pharmacy), Miramistin (pharmacy), Benzalkonium chloride (Sigma Aldrich) were used as reference drug for bacteria.

II. Physical constants and spectral data for alkyl (*N*-alkyl-*N*,*N*-dioctylammoniomethyl)phosphonates 2a-i

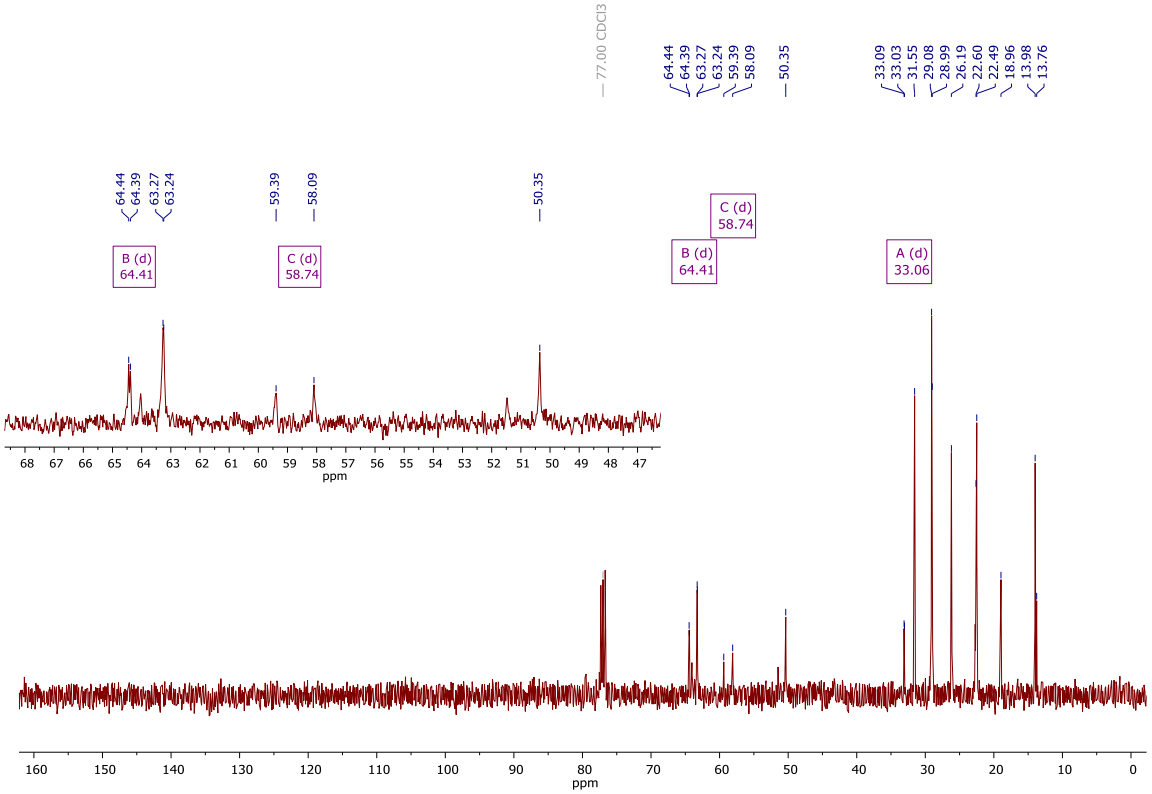
NMR spectra



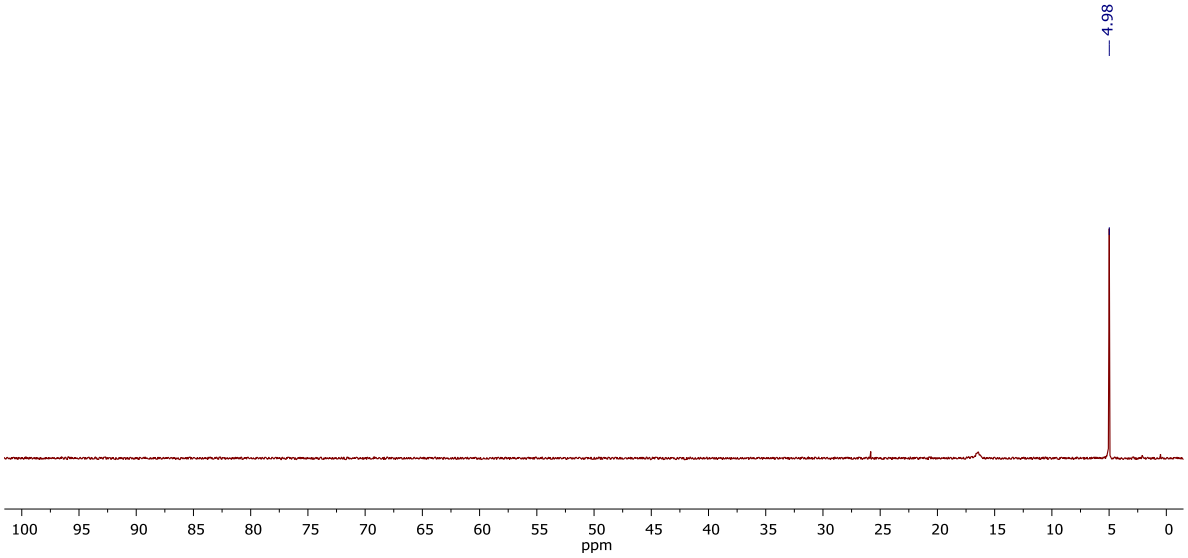
$^{31}P\{^{1}H\}$ NMR (propan-2-ol) of butyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)- phosphonate **2a**



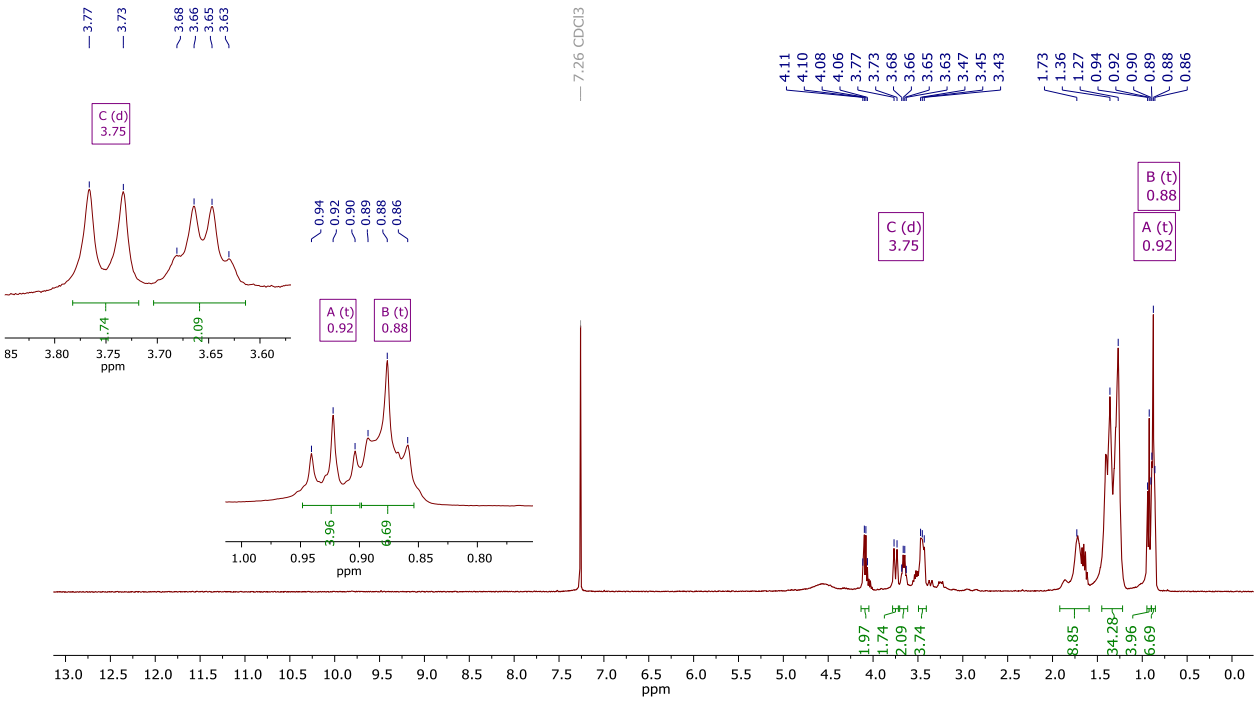
^{1}H NMR ($CDCl_3$) of butyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2a**



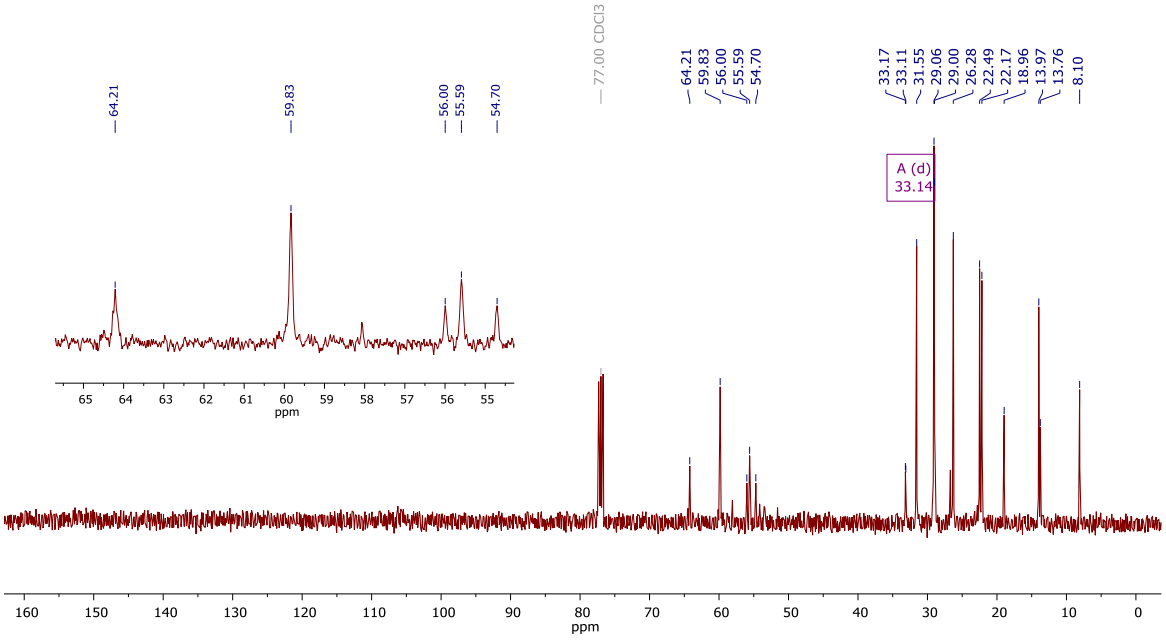
$^{13}C\{^{1}H\}$ NMR ($CDCl_3$) of butyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2a**



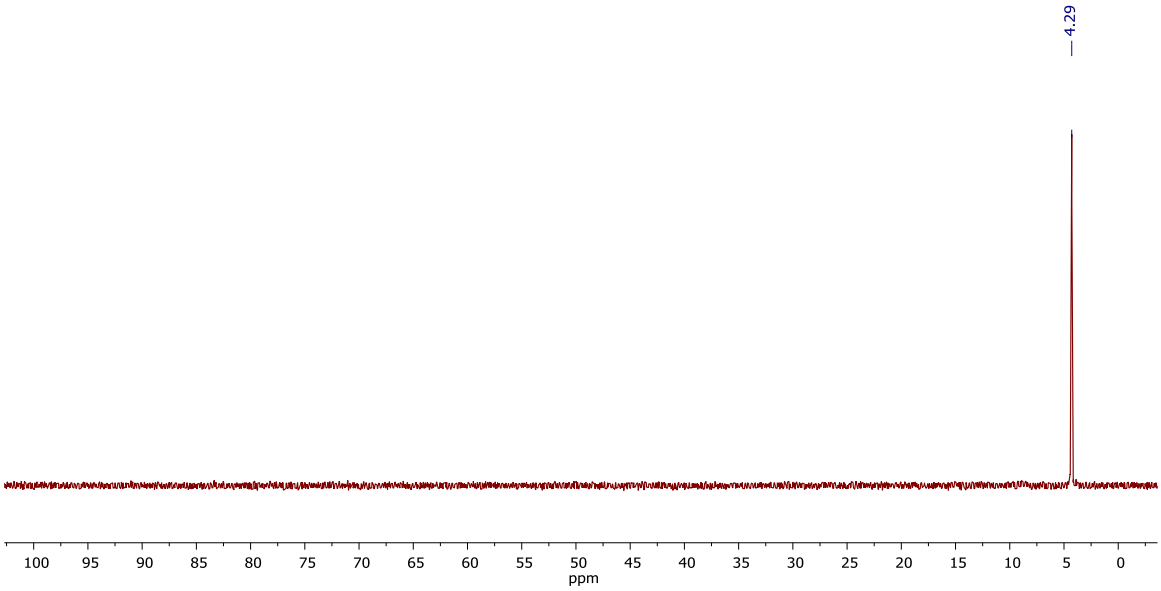
$^{31}P\{^{1}H\}$ NMR (propan-2-ol) of butyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)- phosphonate **2b**



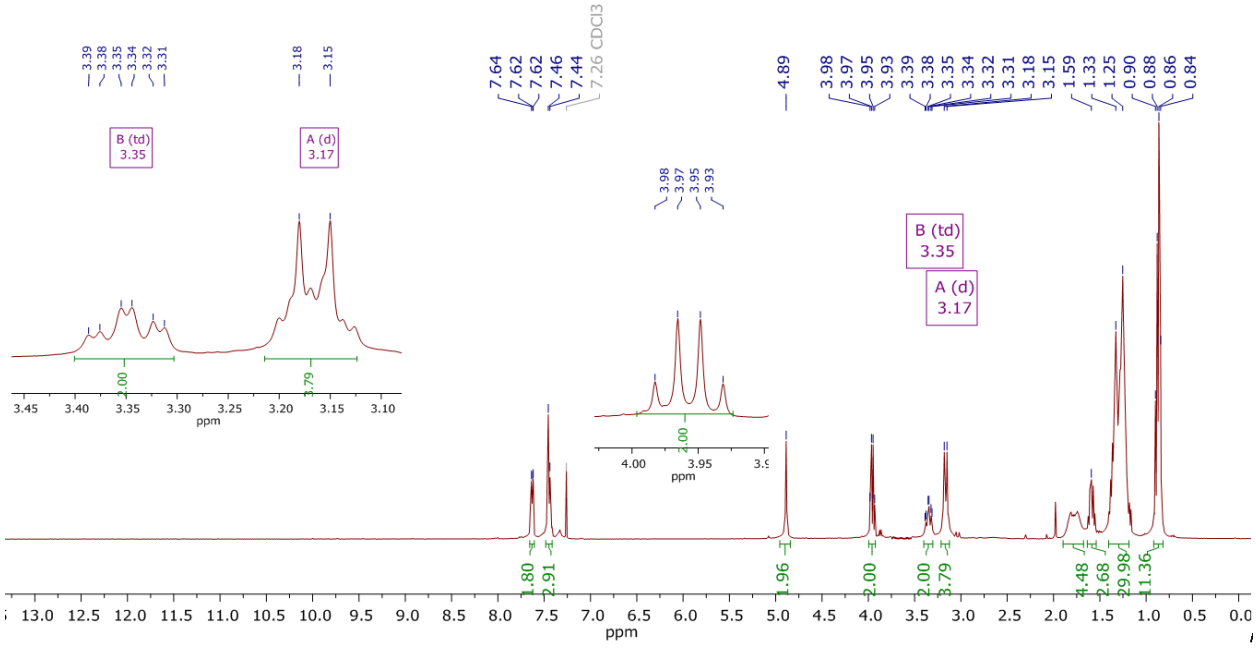
^{1}H NMR ($CDCl_3$) of butyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2b**



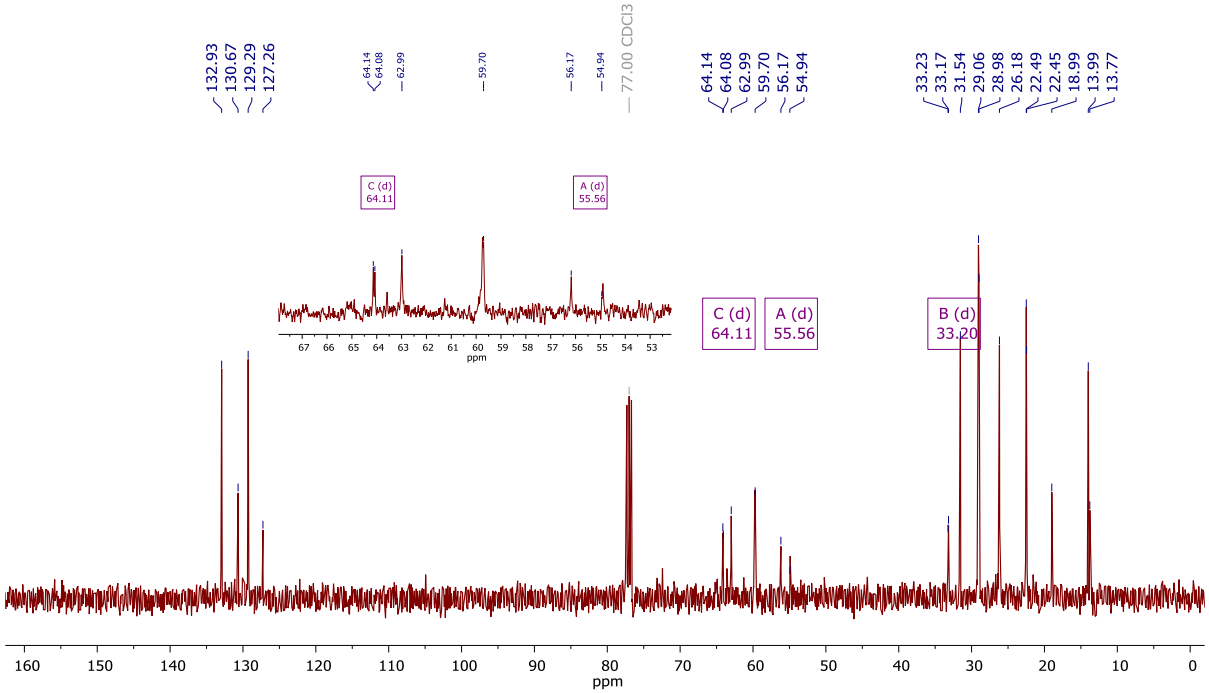
^{13}C NMR ($CDCl_3$) of butyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2b**



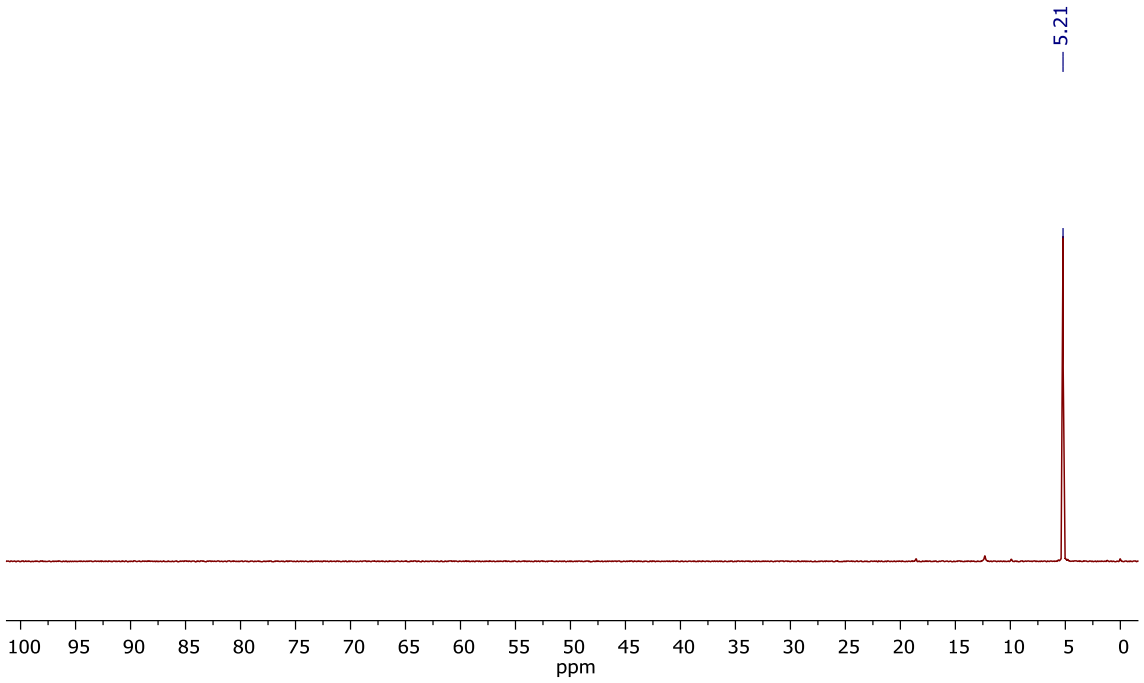
$^{31}P\{^{1}H\}$ NMR (propan-2-ol) of butyl (*N*-benzyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2c**



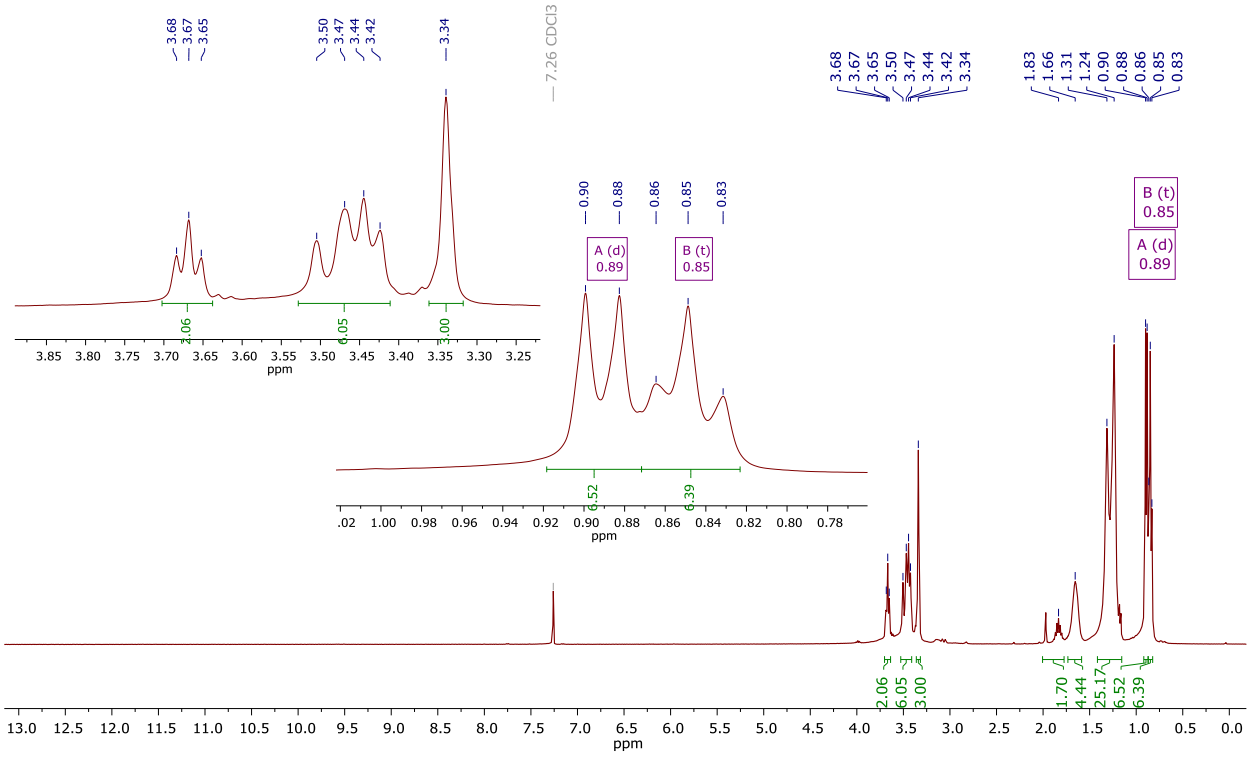
^{1}H NMR ($CDCl_3$) of butyl (*N*-benzyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2c**



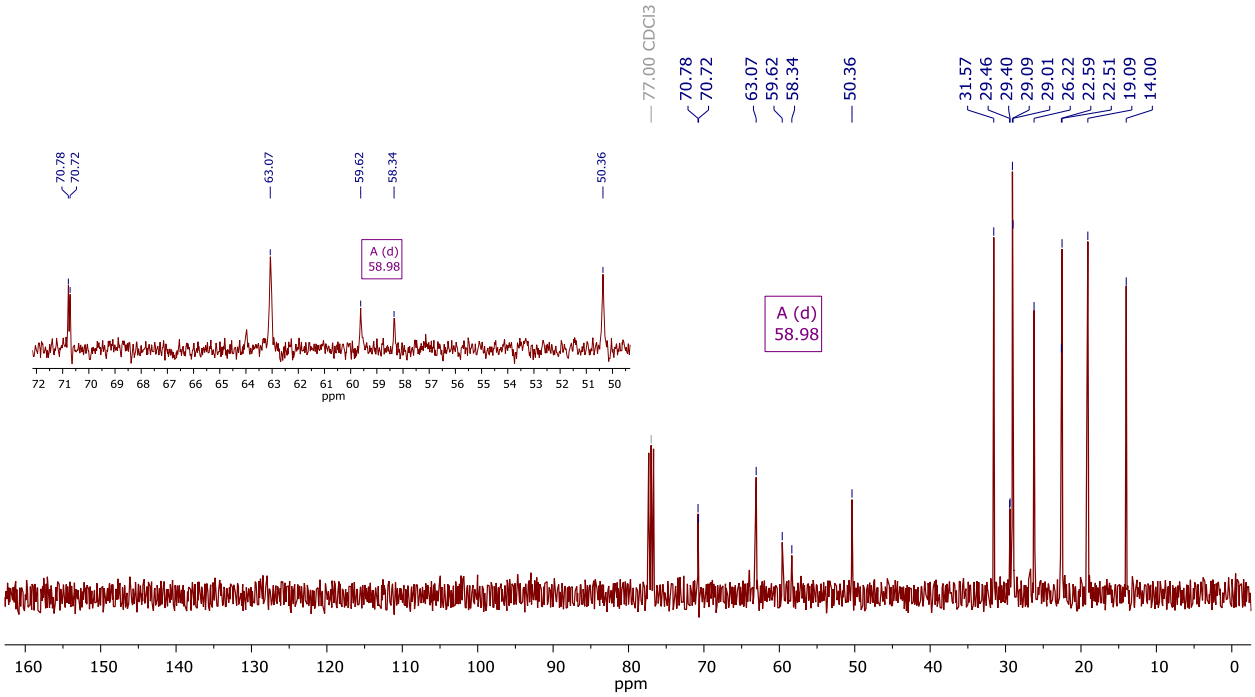
^{13}C NMR ($CDCl_3$) of butyl (*N*-benzyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2c**



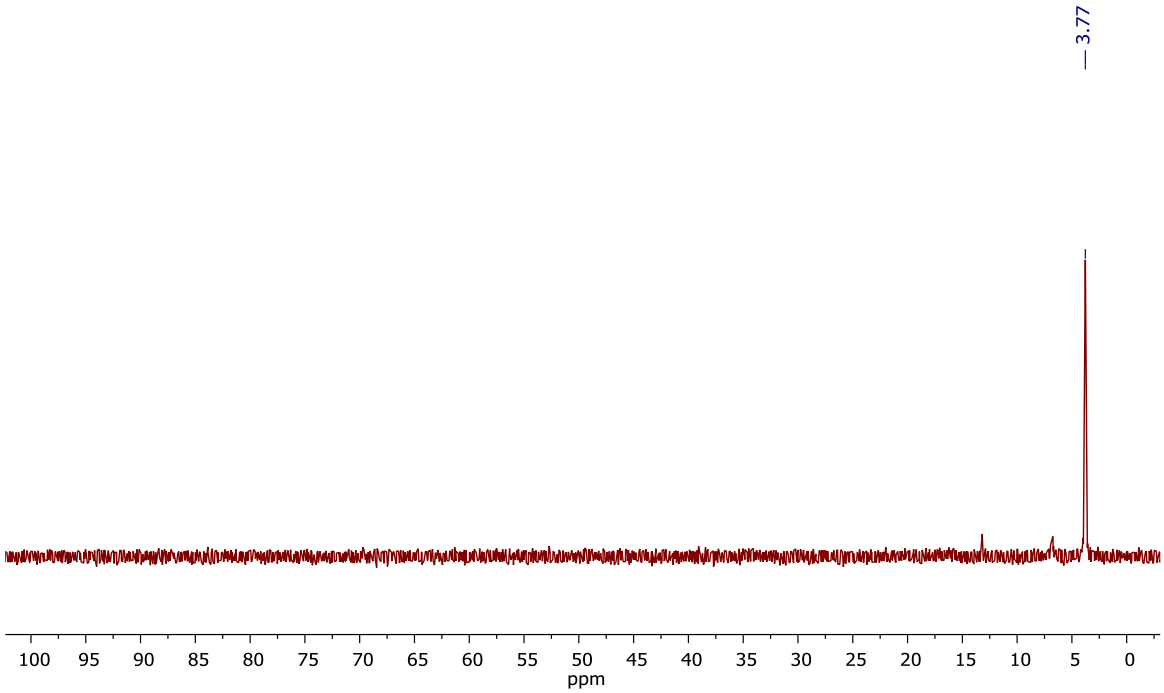
$^{31}P\{^1H\}$ NMR (propan-2-ol) of isobutyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)-phosphonate **2d**



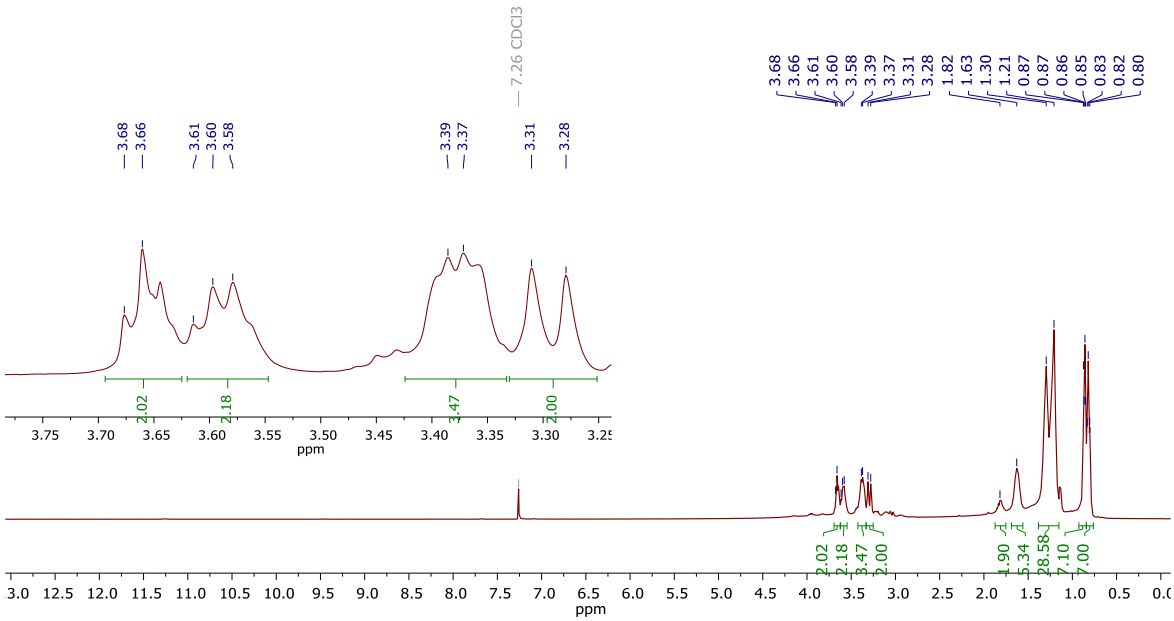
^{1}H NMR ($CDCl_3$) of isobutyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2d**



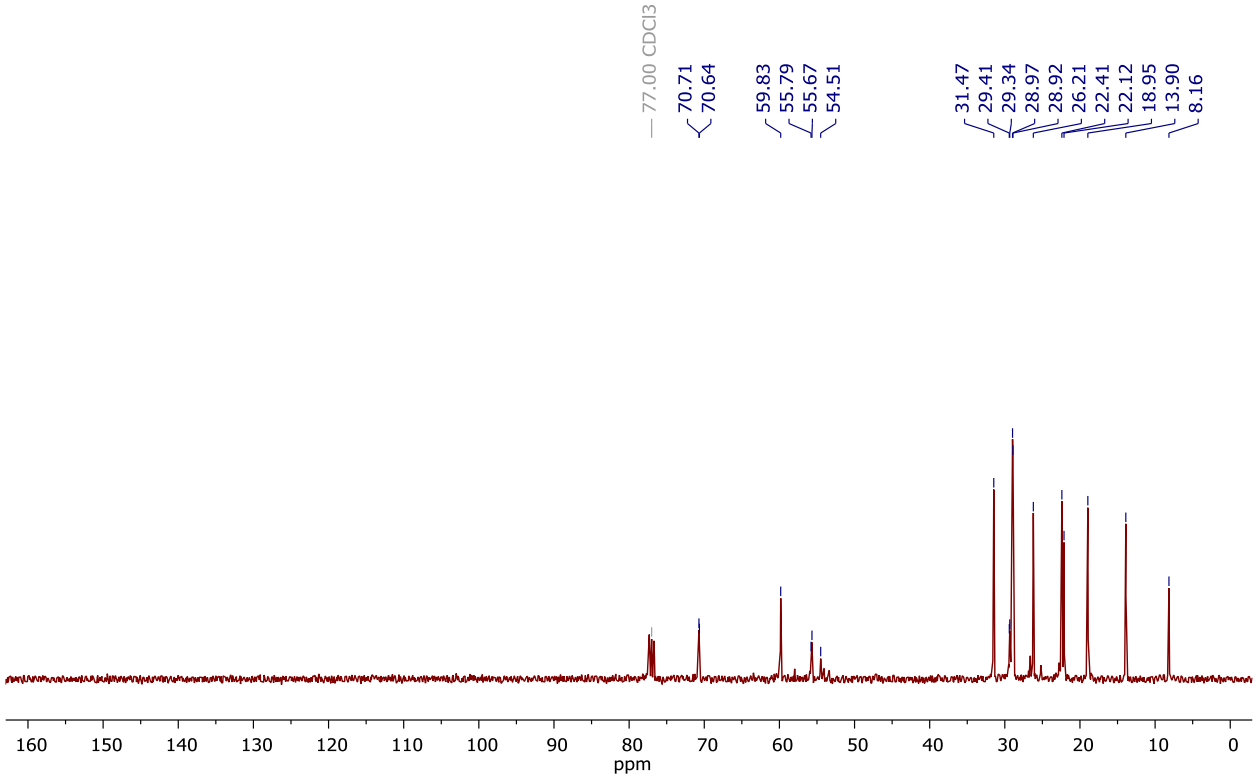
$^{13}C\{^{1}H\}$ NMR ($CDCl_3$) of isobutyl (*N*-methyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2d**



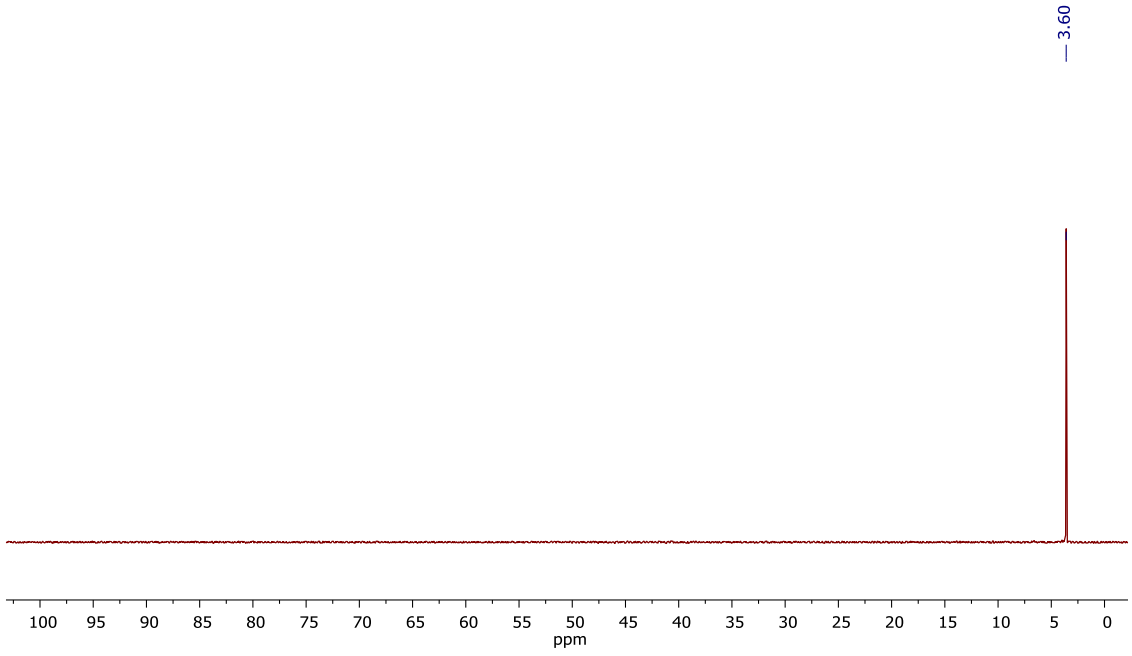
$^{31}P\{^{1}H\}$ NMR (propan-2-ol) of isobutyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)-phosphonate **2e**



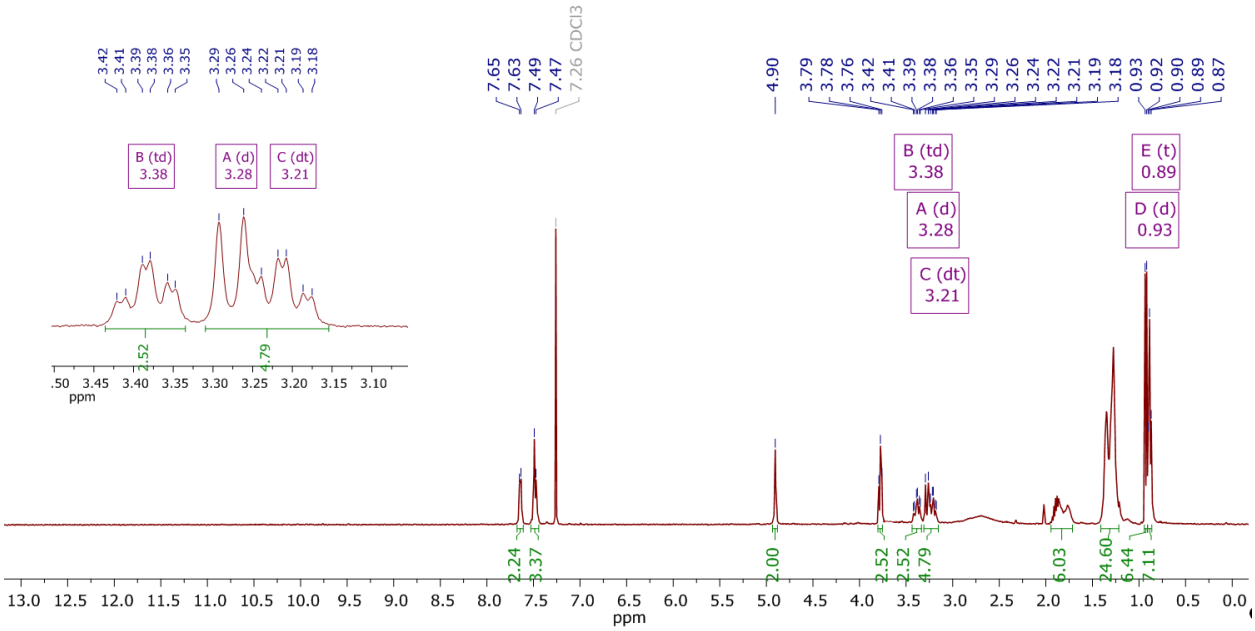
^{1}H NMR ($CDCl_3$) of isobutyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2e**



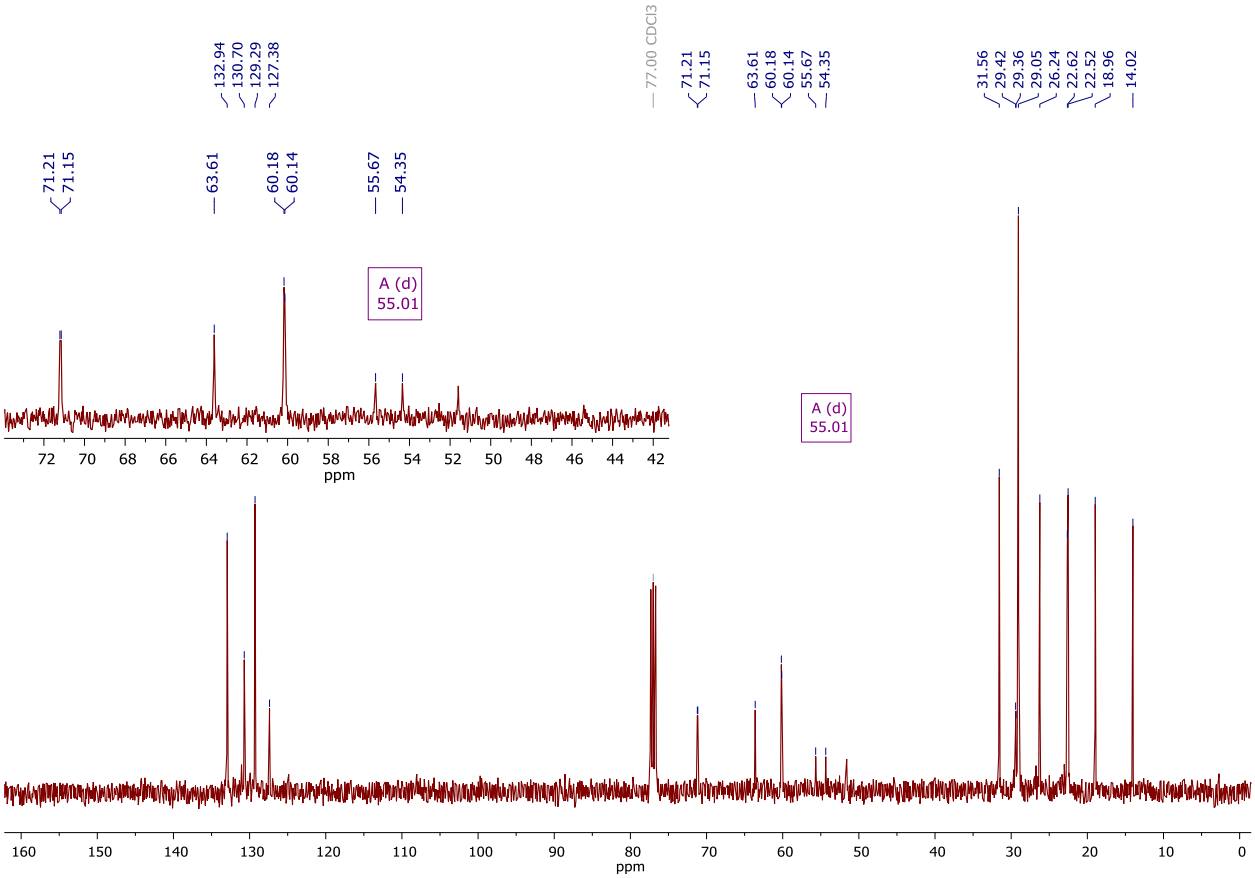
$^{13}C\{^{1}H\}$ NMR ($CDCl_3$) of isobutyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2e**



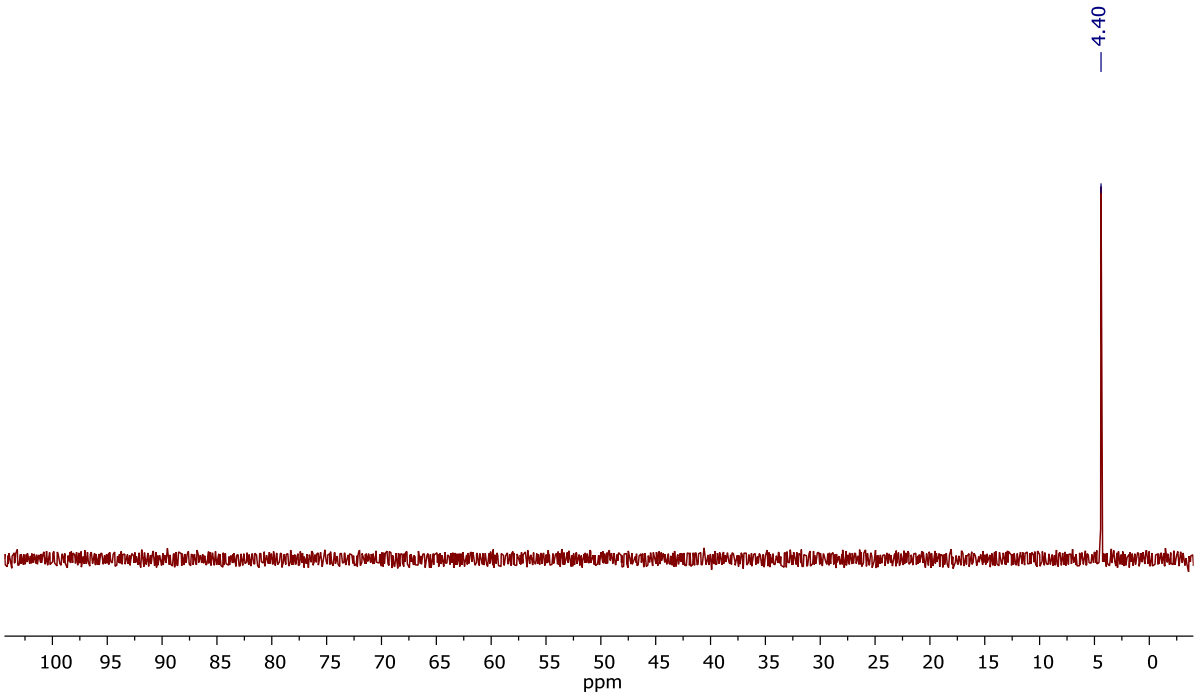
$^{31}P\{^{1}H\}$ NMR (propan-2-ol) of isobutyl (*N*-benzyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2f**



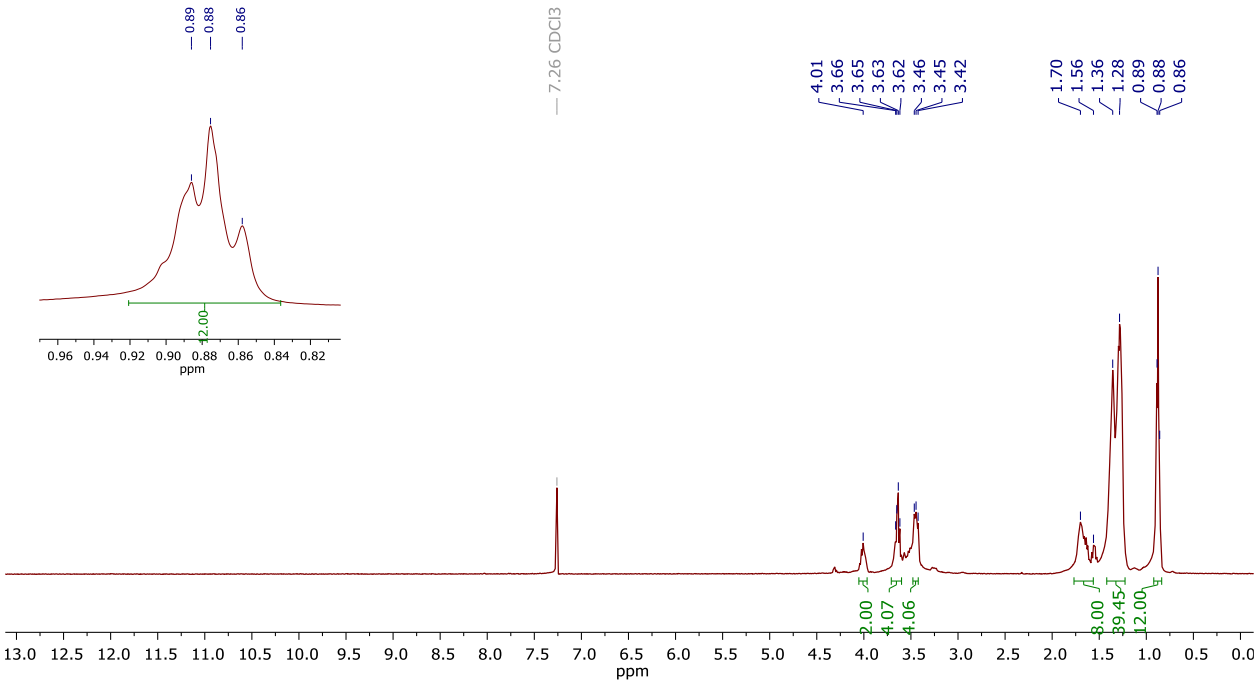
^{1}H NMR ($CDCl_3$) of isobutyl (*N*-benzyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2f**



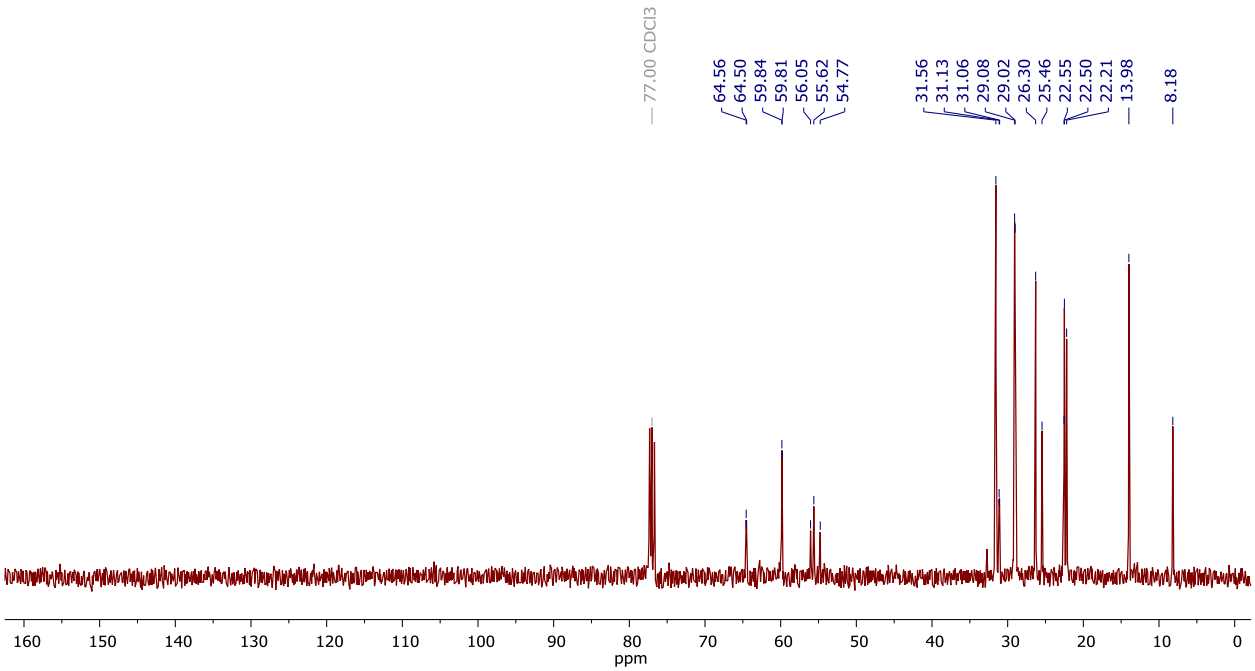
^{13}C{^{1}H} NMR ($CDCl_3$) of isobutyl (*N*-benzyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2f**



$^{31}P\{^{1}H\}$ NMR (propan-2-ol) of hexyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2h**



^{1}H NMR ($CDCl_3$) of hexyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2h**



^{13}C NMR ($CDCl_3$) of hexyl (*N*-ethyl-*N*,*N*-dioctylammoniomethyl)phosphonate **2h**

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S2. A.R. Garifzyanov, N.V. Davletshina, L.I. Akhmadullina, I.T. Safiullin, R. A. Cherkasov. *Russ. J. Gen. Chem.*, 2018, **88**, 2445. (*Zh. Obshch. Khim*., 2018, 88, 1923).