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N-Substituted aziridine-2-phosphonic acids and their antibacterial activities

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Abstract: *N*-substituted aziridine diethyl phosphonates were synthesized easily in two steps starting from vinyl phosphonate or acetyl phosphonate. The controlled mono hydrolysis without opening the aziridine ring under mild reaction conditions was achieved by alkaline solution of LiOH. The corresponding phosphonic acid lithium salt was desalted by the use of Amberlite IRC-50H$^+$ acting as an efficient and recyclable weakly acidic cation exchange promoter. Antimicrobial activity of the synthesized compounds was tested against *E. coli* ATCC 25922, *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae* NRLL B-4420, *Acetobacter baumanii* (wild type), *Pseudomonas aeroginosa* (ATCC 27853) and *Enterobacter aerogenes* NRLL B-3567. In general, all the compounds showed moderate antibacterial activity.

Keywords: Aziridine-2-phosphonic acids; atibacterial activity; hydrolytic cleavage; ion-exchange.

1. Introduction

α-Aminophosphonic acids are considered to be the analogues of natural α-amino acids, the 'building blocks' of peptides and proteins. After the discovery of aminophosphonic acids in living systems, it has stimulated the intensive research interest in synthesizing α-aminophosphonic acids which gave rise to a new class of drugs and other biologically active compounds used as anticancer agents, neuromodulators, antibiotics, enzyme inhibitors, plant growth regulators, herbicides, antibacterial agents, and many other applications.[1-3] Although, the acyclic α-aminophosphonate derivatives have been investigated extensively in terms of their synthesis, characterization, stereochemistry and biological activities,[4-7] only limited studies have been reported for the aziridine diphosphonic acids synthesized by ring closure of acyclic β-aminophosphonic acids.[8-12]

Until our first publication, no systematic study in which aziridine-2-phosphonic acids derived directly from their corresponding diethyl aziridine-2-phosphonates has been reported.[13] In our first publication we have reported the synthesis of a series of aziridine-2-phosphonic acids and the cellular cytotoxicity of these compounds against the HCT-116 colorectal cancer cell lines. Some of these compounds showed higher cytotoxicity than the reference cancer treatment agent etoposide. As a continuation of our previous work, we added three new compounds (**4b**, **4c**, and **4g**) to the previous series and this time looked at their antibacterial activities. This study also allowed us to compare the difference in antibacterial activities of aziridine-2-phosphonates (**3a-l**)[14] and their mono hydrolysis product aziridine-2-phosphonic acids (**4a-l**).

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2. Experimental

2.1. Chemical Material and Apparatus

Unless otherwise mentioned, all reagents were used as received from commercial suppliers. ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Brucker Avance DPX-400 instrument at 400, 100, and 162 MHz, respectively relative to TMS for ^{1}H and ^{13}C NMR and H_3PO_4 for ^{31}P NMR in D_2O. Optical rotations were measured on a Rudolph Research Analytical Autopol III Polarimeter. The mass spectra were obtained with Agilent 6224 High Resolution Mass Time-of-Flight (TOF) LC/MS using an electrospray ionization method and/or HRMS were recorded on a Waters Micromass Q-TOF instrument in ES^+ mode. IR spectra were obtained by Bruker Platinum ATR-IR instruments and are reported in cm^{-1}. Melting points were determined in open-end capillary tubes and are uncorrected. TLC plate was stained with phosphomolybdic acid solution in ethanol. All the aziridine-2-phosphonates **3a**–**l** were prepared as reported previously.[14,23] New aziridine-2-phosphonic acids were also prepared by using the published procedure.[13]

2.2.Chemistry

*2.2.1. Spectroscopic data of ethyl hydrogen (S)-1-((S)-1-phenylethyl)aziridin-2-ylphosphonate (**4b**):* Compound **4b** was obtained as a light yellow solid in 81% yield. R_f = 0.34, acetone/toluene/acetic acid/*n*-butanol/water (1:1:1:1:1); mp: 223 °C; $[\alpha]_D^{35}$ = -80.58 (*c* 3.4×10^{-3}, MeOH); ^{1}H-NMR (400 MHz, D_2O) ppm: 0.74 (t, *J* = 6.4 Hz, 3H), 1.29 (d, *J* = 5.8 Hz, 3H), 1.47–1.56 (m, 1H), 1.70 (t, *J* = 6.8 Hz, 1H), 1.90–1.97 (m, 1H), 2.43 (q, *J* = 6.1 Hz, 1H), 3.10–3.27 (m, 2H), 7.19–7.30 (m, 5H); ^{13}C-NMR (100 MHz, D_2O) ppm: 15.92 ($OCH_2\underline{C}H_3$, d, J_{C-P} = 5.9 Hz), 21.54 ($\underline{C}H_3$), 31.47 ($\underline{C}H_2$-aziridine), 32.86 ($\underline{C}$HP, d, J_{C-P} = 198.1 Hz), 61.11 ($CH_3\underline{C}H_2OP$, d, J_{C-P} = 4.6 Hz), 70.29 ($N\underline{C}HPh$, d, *J* = 4.8 Hz), 127.33 (Ph), 127.81 (Ph), 128.81(Ph), 143.58 (Ph); ^{31}P-NMR (D_2O) ppm:17.74; IR (neat, cm^{-1}) 3285 (broad), 3027 (vw), 2976 (w), 2928 (vw), 1654 (w), 1600 (w), 1225 (s), 1198 (s), 1159 (m), 1075 (s), 1044 (vs), 945 (s); ESI-MS (*m/z*): calculated for $C_{12}H_{18}NO_3P$ $[M-1]^-$: 255.1024 and found 255.1019.

*2.2.2. Spectroscopic data of ethyl hydrogen (R)-1-((S)-1-(naphthalen-2-yl)ethyl)aziridin-2-ylphosphonate (**4c**):* Compound **4c** was obtained as a pale yellow gum in 96% yield. R_f = 0.20, acetone/toluene/acetic acid/*n*-butanol/water (1:1:1:1:1); $[\alpha]_D^{36}$ = -38.5 (*c* 2×10^{-3}, MeOH); ^{1}H-NMR (400 MHz, D_2O) ppm: 1.18 (t, *J* = 7.1 Hz, 3H), 1.46 (d, *J* = 6.5 Hz, 3H), 1.65 (t, *J* = 7.3 Hz, 1H), 1.73 (ddd, *J* = 15.3, 7.2, 4.5 Hz, 1H), 1.80 (dd, *J* = 8.3, 5.0 Hz, 1H), 2.68 (q, *J* = 6.1 Hz, 1H), 3.86–3.99 (m, 2H), 7.44–7.50 (m, 3H), 7.79–7.85 (m, 4H); ^{13}C-NMR (100 MHz, D_2O) ppm: 16.23 ($OCH_2\underline{C}H_3$, d, J_{C-P} = 5.6 Hz), 21.33 ($\underline{C}H_3$), 30.76 ($\underline{C}H_2$-aziridine, d, *J* = 4.0 Hz), 34.51 (CHP, d, J_{C-P} = 199.0 Hz), 61.47 ($CH_3\underline{C}H_2OP$, d, J_{C-P} = 5.2 Hz), 69.96 ($N\underline{C}HNp$, d, *J* = 3.4 Hz), 125.05 (Np), 125.30 (Np), 125.86 (Np), 126.20 (Np), 127.55 (Np), 127.82 (Np), 128.810 (Np), 132.48 (Np), 133.807 (Np), 140.96 (Np); ^{31}P-NMR (D_2O) ppm: 17.65; IR (neat, cm^{-1}) 3354 (broad), 3055 (vw), 2974 (w), 2929 (vw), 2900 (vw), 1600 (m), 1197 (m), 1164 (w), 1072 (s), 1044 (vs), 942 (s); ESI-MS (*m/z*): calculated for $C_{16}H_{20}NO_3P$ $[M+Na]^+$: 305.1181 and found 305.1176.

*2.2.3. Spectroscopic data of ethyl hydrogen (R)-1-((R)-1-cyclohexylpropyl)aziridin-2-ylphosphonate (**4g**):* Compound **4g** was obtained as light yellow gum in 80% yield. R_f = 0.47, acetone/toluene/acetic acid/*n*-butanol/water (1:1:1:1:1); $[\alpha]_D^{36}$ = -20.71 (*c* 8.83×10^{-3}, MeOH); ^{1}H-NMR (400 MHz, D_2O) ppm: 0.75-1.20 (m, 13H), 1.21–1.30 (m, 2H), 1.40–1.65 (m, 4H), 1.70–1.75 (m, 1H), 1.80–1.85 (m, 1H), 3.75–3.90 (m, 2H); ^{13}C-NMR (100 MHz, D_2O) ppm: 16.14 ($OCH_2\underline{C}H_3$, d, *J* = 5.9 Hz), 17.37 ($\underline{C}H_3$), 26.04 (Cy), 26.13 (Cy), 26.26 (Cy), 29.21 & 30.12 (2 × $\underline{C}H_2$-Cy), 31.55 ($\underline{C}$HP, d, J_{C-P} = 200.3 Hz), 33.19 ($\underline{C}H_2$-aziridine, d, *J* = 4.7 Hz), 43.71 ($\underline{C}$H-Cy), 61.36 ($CH_3\underline{C}H_2OP$, d, J_{C-P} = 5.6 Hz), 70.95 ($N\underline{C}HCy$, d, *J* = 5.4 Hz); ^{31}P-NMR (D_2O) ppm:18.96; IR (neat, cm^{-1}) 3285 (broad), 2975 (w), 2922 (m), 2851 (w), 1600 (m), 1448 (w), 1223 (m), 1189 (s) 944 (m), 1046 (vs), 772 (s); ESI-MS (*m/z*): calculated for $C_{12}H_{24}NO_3P$: $[M-1]^-$: 261.1494 and found 261.1501.

2.3. Antibacterial Activity Assays

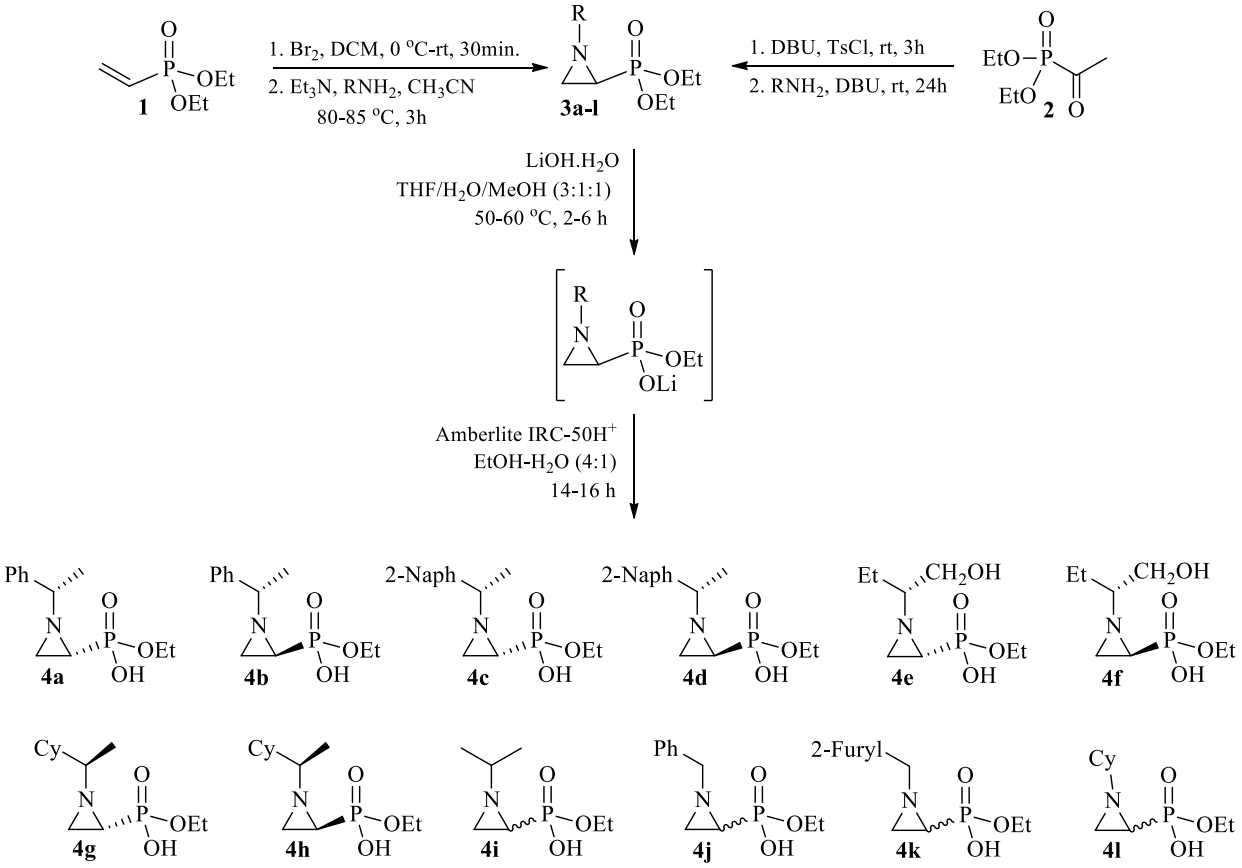
Antibacterial activities of the synthesized compounds were tested against *E. coli* ATCC 25922, *Staphylococcus aureus* OGU no 1. (MRSA), *Klebsiella pneumoniae* NRLL B-4420, *Acetobacter baumanii* (wild type), *Pseudomonas aeroginosa* (ATCC 27853) and *Enterobacter aerogenes* NRLL B-3567 were expressed as the minimum inhibitory concentration (MIC). The standard bacteria strains were obtained from the U.S. Department of Agricultural Service, Peoria, IL). *A. baumanii* and *S. aureus* were obtained from the Osmangazi University Hospital in Eskisehir-Turkey.

The MIC values were determined by the microdilution testing protocol.[25] The stock solutions of the compounds were prepared in DMSO. Chloramphenicol was used as the standard antibacterial agent. The observed data on the antimicrobial activity of the compounds and the control drugs was reported as the MIC values, in µg/mL.

3. Results and Discussions

3.1. Chemistry

Our laboratory is systematically investigating the synthesis and biological activities of acyclic α-aminophosphonates and aziridine-2-phosphonate derivatives. Their synthesis proceeds through commercially available diethyl vinyl phosphonate **1** via the Gabriel-Cromwell reaction or from acetyl phosphonate **2**, developed by our group as shown in Scheme 1. Both are two step synthesis providing aziridine-2-phosphonates in good yields.[14]



Scheme 1. Synthesis of aziridine-2-phosphonic acids

In the course of our investigations on the synthesis of aziridine derived phosphonic acid monoesters, after exhaustive experiments, we discovered that *N*-functionalized, non-activated aziridines

offer an uncommon combination of reactivity. They are not only sensitive to acidic conditions such as trans silylation procedure or acid hydrolysis[15] but also undergo aziridine ring opening reaction with nucleophilic reagents such as metal halides even in the absence of acid.[16-20]

On the other hand, during desalting step of phosphonic acid lithium salt, strongly acidic cation-exchangers such as Amberlite IR-120H^+ or Dowex 50H^+ aziridine ring opening took place more likely through the protonation of the aziridine nitrogen.[21] These in turn prompted us to use suitable cationic exchanger which will not only preserves the aziridine ring but will also smoothen the process of the ion exchange. It is noteworthy to mention that lithium ion exhibits a particularly high affinity for H^+ from the cationic exchangers having carboxylic functional group like Amberlite IRC-50 H^+ as compared to K^+ and Na^+ which exhibit high affinity for H^+ from the cationic exchangers having sulphonic acid functional group like Amberlite IR-120H^+ or Dowex 50 H^+.[22] For this reason LiOH.H_2O was used as a base to execute the hydrolysis rather than NaOH or KOH.

The mono hydrolysis of diethyl aziridine 2-phosphonates to corresponding phosphonic acids was achieved in 2-6 hours at 60 ^oC in a mixed solvent system (THF/H_2O/MeOH; 3:1:1). The progress of the reaction was followed easily by the tlc control. After removing THF and MeOH, desalting process was achieved by stirring the lithium salt with Amberlite IRC 50H^+ for 16-18 h in EtOH-H_2O (4:1) solvent system. It was observed that desalting could not be completed at shorter times and also it was necessary to use ~70-fold excess of the cation exchanger.

New phosphonic acid derivatives **4b**, **4c**, and **4g** were characterized on the basis of their spectral data, especially C–P spin coupling ($^1J_{C\text{-}P}$ = 194–200 Hz) of the C-2 carbon in ^{13}C NMR spectra was noteworthy. The presence of a broad peak around 3300 cm^{-1} in the IR spectra of α-hydroxyaziridinephosphates clearly indicated the presence of the P–OH group. The characteristic signature of the P=O bond appears around 1199 cm^{-1}, whereas the peaks around 1074 cm^{-1} and 945 cm^{-1} corresponded to the symmetric and anti-symmetric P–O–C stretching vibrations.[24]

3.2. Antimicrobial Activity

MICs were recorded as the minimum concentration of a compounds that inhibits the growth of tested microorganisms (Table 1).

Table 1. Antibacterial activities of the synthesized compounds **4a-4l***

Compound	*E. coli*	*S. aureus*	*K. pneumoniae*	*A. baumanii*	*P.aeroginosa*	*E. aerogenes*
4a	250	250	250	250	250	250
4b	250	500	250	250	250	250
4c	250	250	250	250	250	250
4d	250	250	250	250	250	250
4e	250	250	250	250	250	250
4f	250	250	250	250	250	250
4g	250	250	125	125	250	250
4h	250	250	125	250	250	250
4i	250	250	250	250	250	250
4j	250	250	250	250	250	250
4k	250	250	250	250	250	250
4l	250	250	250	250	250	250
CHL	3.9	3.9	1.95	125	250	15.6

*MIC values (μg/mL); CHL: chloramphenicol

In general, the antibacterial assessments revealed that all of the compounds showed moderate activity against the tested bacteria. The MIC values were generally 250 μg/mL against all strains tested. It is worth mentioning that all the compounds showed the same activity against *Pseudomonas aeroginosa* as the reference antibiotic chloramphenicol (CHL). For the *Acetobacter baumanii* only compound **4g** showed the same activity as the reference antibiotic CHL, the others were less effective. On the other hand, *Pseudomonas aeroginosa* was the most resistant bacterium against the antibiotic.

4. Conclusion

Antibacterial activity of twelve aziridine-2-phosphonic acids were tested for the first time in the literature by measuring their MIC values. They all showed moderate activity against the tested bacteria. Comparing the antibacterial activity of diethyl aziridine-2-phosphonates (ref 23) to aziridine phosphonic acids (this study), phosphonates showed higher activity than their phosphonic acid derivatives. It is also important to indicate that disk diffusion method was used for the phosphonate series published before.

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Supporting Information

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References

[1] Naydenova, E. D.; Todorov, P T.; Troev, K. D. Recent synthesis of aminophosphonic acids as potential biological importance. *Amino Acids* **2010**, *38*, 23–30.
[2] Horsman, G. P.; Zechel, D. L. Phosphonate biochemistry. *Chem. Rev*. **2017**, *117*, 5704−5783.
[3] Rabinowitz, R. Synthesis of monoesters of phosphonic acids. *J. Am. Chem. Soc.*, **1960**, *82*, 4564–4567.
[4] Huang, J.; Chen, R. An overview of recent advances on the synthesis and biological activity of α-aminophosphonic acid derivatives. *Heteroatom Chem*. **2000**, *11*, 480-492.
[5] Failla, S.; Finocchiaro, P.; Consiglio, G. A. Syntheses, characterization, stereochemistry and complexing properties of acyclic and macrocyclic compounds possessing α-amino or α-hydroxyphosphonate units. *Heteroatom Chem*. **2000**, *11*, 493-504.
[6] Zefirov, N. S.; Matveeva, E. D. Catalytic Kabachnik-Fields reaction: new horizons for old reaction. *Arkivoc* **2008**, (*i*), 1-17.
[7] Tarik E. A. Synthetic methods of cyclic α-aminophosphonic acids and their esters. *Arkivoc* **2014**, *(i)*, 21-91.
[8] Christensen, B. G.; Beattie, T. R. Ger. Offen. 2011092, **1970**; Chem. Abstr. **1971**, *74*, 42491.
[9] Davis, F. A.; Wu, Y. Z.; Yan, H.; Kavirayani, R. P.; McCoull, W. 2*H*-Azirine 3-phosphonates: A new class of chiral iminodienophiles. asymmetric synthesis of quaternary piperidine phosphonates. *Org. Lett.* **2002**, *4*, 655-658.
[10] Davis, F. A.; McCoull, W.; Titus, D. D. Asymmetric synthesis of α-methylphosphophenylalanine derivatives using sulfinimine-derived enantiopure aziridine-2-phosphona. *Org. Lett.* **1999**, *1*, 1053-1055.
[11] Davis, F. A.; Wu, Y.; Yan, H.; McCoull, W.; Prasad, K. R. Asymmetric synthesis of aziridine 2-phosphonates from enantiopure sulfinimines (*N*-sulfinyl imines). Synthesis of α-amino phosphonates. *J. Org. Chem*. **2003**, *68*, 2410-2419.
[12] Zygmunt, J. Aziridine-2-phosphonic acid, the valuable synthon for synthesis of 1-amino-2-functionalized ethanephosphonic acids. *Tetrahedron* **1985**, *41*, 4979-4982.
[13] Khan, R.; Ulusan, S.; Banerjee, S.; Dogan, Ö. Synthesis, characterization and evaluation of cytotoxic activities of novel aziridinyl phosphonic acid derivatives. *Chem. Biodiversity* **2019**, *16*, e1900375.
[14] Doğan, Ö.; Babiz, H.; Gözen, A. G.; Budak, S. Synthesis of 2-aziridinyl phosphonates by modified Gabriel-Cromwell reaction and their antibacterial activities. *Eur. J. Med. Chem*. **2011**, 46, 2485-2489.
[15] Stanković, S.; D'hooghe M.; Catak, S.; Eum H.; Waroquier, M.; Speybroeck, V. V.; De Kimpe, N.; Ha, H. J. Regioselectivity in the ring opening of non-activated aziridines. *Chem. Soc. Rev.*, **2012**, ***41***, 643-665.
[16] Florio, S.; Luisi, R. Aziridinyl anions: Generation, reactivity, and use in modern synthetic chemistry. *Chem. Rev*. **2010**, *110*, 5128-5157.

[17] Lu, P. Recent developments in regioselective ring opening of aziridines. *Tetrahedron* **2010**, *66*, 2549-2560.
[18] Hu, X. E. Nucleophilic ring opening of aziridines. *Tetrahedron* **2004**, *60*, 2701-2743.
[19] Righi, G.; Potini, C., Bovicelli, P. Stereo- and regioselective ring opening of alkenyl aziridines with metal halides. *Tetrahedron Lett*. **2002**, *43*, 5867-5869.
[20] Righi, G., Franchini, T.; Bonini, C. Highly regioselective opening of optically active N-Boc-2,3-aziridino alcohol derivatives with metal halides. *Tetrahedron. Lett.* **1998**, *39*, 2385-2388.
[21] Hu, X. E. Nucleophilic ring opening of aziridines. *Tetrahedron* **2004**, *60*, 2701–2743.
[22] Ion Exchange Technologies, edited by Kilislioglu, A. Intech Open, November, 2012.
[23] Dogan, Ö.; Polat-Çakır, S.; Beksultanova N.; Altanlar N.; Simsek D.; Karabıyık H. Enantioselective synthesis of new chiral 2-aziridinyl phosphonates and studies of their biological activities. *Tetrahedron: Asymmetry* **2017**, *28*, 324–329.
[24] Hossain, S.; Gupta, K. S.; Murugavel, R. 2,6-Dimethylphenol derived H-phosphonate and α-hydroxyphosphonate: facile synthesis, crystal chemistry, supramolecular association and metal complexation. *Cryst. Eng. Comm*. **2015**, *17*, 4355.
[25] Qaiyumi, S. Macro-and microdilution methods of antimicrobial susceptibility testing. In "Antimicrobial Susceptibility Testing Protocols", Schwalbe, R., Steele-Moore, L. and Goodwin, A. C. (eds), p.75-79, 2007, CRC Press.