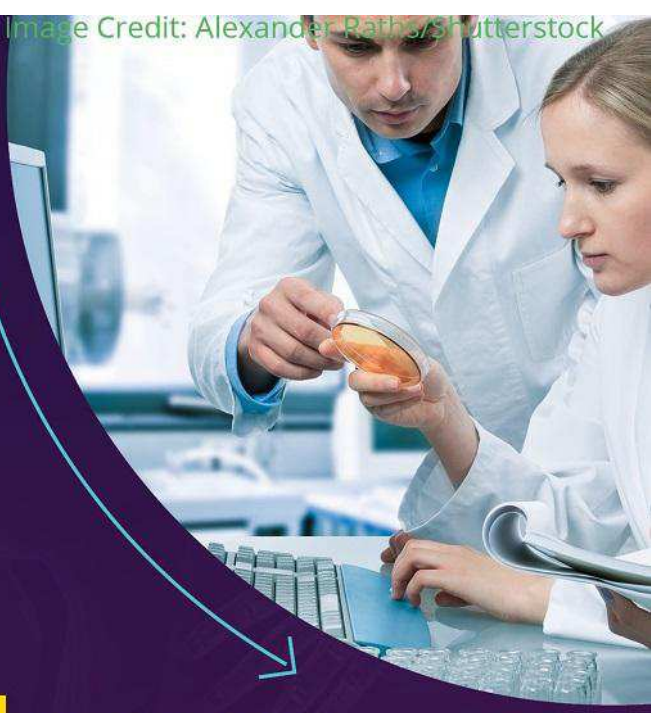
Application Note



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Research Article

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Fragmentation pathways and structural characterization of organophosphorus compounds related to the Chemical Weapons Convention by electron ionization and electrospray ionization tandem mass spectrometry

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RATIONALE: For unambiguous identification of Chemical Weapons Convention (CWC)-related chemicals in environmental samples, the availability of mass spectra, interpretation skills and rapid microsynthesis of suspected chemicals are essential requirements. For the first time, the electron ionization single quadrupole and electrospray ionization tandem mass spectra of a series of *O*-alkyl *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidates (Scheme 1, cpd **4**) were studied for CWC verification purposes.
METHODS: *O*-Alkyl *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidates were prepared through a microsynthetic method and were analyzed using electron ionization and electrospray ionization mass spectrometry with gas and liquid chromatography, respectively, as MS-inlet systems. General EI and ESI fragmentation pathways were proposed and discussed, and collision-induced dissociation studies of the protonated derivatives of these compounds were performed to confirm proposed fragment ion structures by analyzing mass spectra of deuterated analogs.
RESULTS: Mass spectrometric studies revealed some interesting fragmentation pathways during the ionization process, such as McLafferty rearrangement, hydrogen rearrangement and a previously unknown intramolecular electrophilic aromatic substitution reaction.
CONCLUSIONS: The EI and ESI fragmentation routes of the synthesized compounds **4** were investigated with the aim of detecting and identifying CWC-related chemicals during on-site inspection and/or off-site analysis and toxic chemical destruction monitoring.

During the Iran-Iraq war (1980–1988), the Iraqi forces employed chemical warfare agents (CWAs) and in 1995 a Japanese cult utilized Sarin, a chemical nerve agent, to attack civilians in the Tokyo subway system.[1,2] These real and tragic events point to the great threat of chemical weapons. Therefore, there has been a worldwide concern to eliminate the presence of chemical weapons. In the investigation of chemical warfare terrorism and the pursuit of disarmament activities, the detection of CWAs is an important task to protect both civilians and security personnel.[3–8] To ensure that the Chemical Weapons Convention (CWC) treaty is respected, the detection of trace levels and the identification of CWC-related compounds are required in a variety of environmental samples.[9–11] Gas or liquid chromatography (GC or LC) coupled with mass spectrometry (MS) is widely used for the analysis of CWAs. GC/MS analysis is typically performed under electron ionization (EI) conditions, and the EI mass spectra of many CWC-related compounds are available through the Organization for the Prohibition of Chemical Weapons Central Analytical Database (OCAD), thus facilitating the identification of CWC-related compounds.[12,13] LC coupled with electrospray ionization (ESI)-MS is the preferred method for the analysis of polar compounds.[14,15]

Nerve agents are a class of highly reactive organophosphorus compounds that mainly target the central nervous system. These extremely potent chemical weapons are organophosphate acetylcholinesterase inhibitors (AChE), which hydrolyze and terminate the action of the neurotransmitter acetylcholine. Inhibition of AChE results in overstimulation of the cholinergic nerves, leading to respiratory paralysis and finally death.[16,17] Alkyl *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidates, a class of organophosphorus compounds, are included in Schedule 2.B.04 of the CWC, and, to the best of our knowledge, no detailed investigation of the mass fragmentation of these compounds has been published. Herein, we wish to report a general microsynthetic procedure for these

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compounds (Scheme 1) along with possible MS fragmentation pathways elucidated from EI-MS and ESI-MS/MS data. The fragmentation pathways were confirmed by performing MS/MS on deuterated analogs.

EXPERIMENTAL

Reagents and chemicals

All the chemicals required for the microsynthesis of *O*-alkyl *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidates were purchased from Sigma-Aldrich (St. Louis, MO, USA), Fluka (Neu-Ulm, Germany), and Merck (Darmstadt, Germany), and were used as received. Methylphosphonic difluoride (Scheme 1, cpd **1**) was synthesized by use of a method described elsewhere.[18] Isopropanol-d_6 was prepared by reduction of acetone-d_6 by sodium borohydride.[19]

GC/MS analysis

GC/MS analyses were performed using an Agilent 6890 N gas chromatograph equipped with a 5973 quadrupole mass selective detector (MSD; Agilent Technologies, Inc., Santa Clara, CA, USA), a HP-5MS (5% phenyl, 95% dimethylpolysiloxane, Agilent's J&W Scientific) capillary column (30 m, 320 mm i.d. and 0.25 mm film thickness), and helium as the carrier gas at constant flow rate of 1.8 mL min^{-1}. The oven temperature was set at 40 °C for 3 min, then ramped to 280 °C at 10 °C/min and held for 6 min. The samples were injected in splitless mode at an injection temperature of 250 °C. The temperatures of the EI source and analyzer were kept at 230 and 150 °C, respectively. The scan range was *m/z* 35–500.

LC/MS/MS analyses

LC/MS/MS analysis was performed on an 1200 LC system (Agilent, Waldbronn, Germany) coupled to an Agilent 6410 triple quadrupole tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The LC column was an Agilent rapid resolution HT Zorbax SB-C18 (3 × 150 mm, 3.5 µm; Agilent Technologies, Santa Clara, CA, USA). The column temperature was set at 25 °C. A gradient elution was applied using 20 mM formic acid solution in water as solvent (A) and 20 mM formic acid solution in acetonitrile as solvent (B). The initial condition was set at 10% of B with a 10 min hold. The following solvent gradient was applied: from 95% A and 5% B to 5% A and 95% B within 50 min, hold for 10 min. The flow rate was set at 0.1 mL min^{-1} and 10 µL of samples were injected into the instrument using an autosampler. The ESI and fragmentor voltages were set at 4000 and 70 V, respectively. The heated capillary temperature was maintained at 300 °C. The drying gas (nitrogen) flow rate and nebulizer gas pressure were 10 L min^{-1} and 40 psi, respectively. MS/MS product ion scans were carried out at a collision energy of 22–25 eV. Ultra-high-purity nitrogen was used as the nebulizer and collision gas. The EI-MS, ESI-MS and ESI-MS/MS spectra of cpd **4** are included in the Supporting Information.

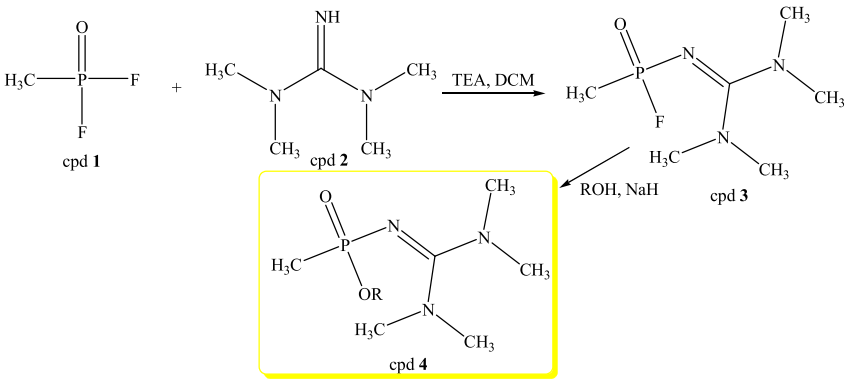
NMR analysis

An Avance DRX-250 MHz NMR spectrometer (Bruker BioSpin, Rheinstettten, Germany) was employed for ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR experiments. All the spectra were recorded at ambient temperature using $CDCl_3$ as the solvent.

General procedure for microsynthesis of cpds 3 and 4

N-[Bis(dimethylamino)methylidene]-*P*-methylphosphonamidic fluoride (**3**) was synthesized by the controlled addition of *N,N,N′,N′*-tetramethylguanidine to a solution of methylphosphonic difluoride (**1**). *N,N,N′,N′*-Tetramethylguanidine (0.60 mmol) and triethylamine (0.65 mmol) in dichloromethane (DCM) (500 µL) were added slowly into the solution of methylphosphonic difluoride (0.40 mmol) in DCM (500 µL), while stirring at 0–5 °C. After 30 min, the resulting precipitate was filtered off and the solution was analyzed by GC/MS.

The corresponding *O*-alkyl *N*-[bis(dimethylamino)-methylidene]-*P*-methylphosphonamidates **4** were synthesized by the addition of ROH to a solution of cpd **3**. Sodium hydride powder (NaH, 60% in mineral oil, 0.5 mmol) was washed with dry hexane twice. The hexane was removed and DCM (500 µL) was added and the solution was kept stirring at 0–5 °C. The appropriate alcohol (0.5 mmol) was slowly added into the suspension and the reaction solution



Scheme 1. General method for the microsynthesis of *O*-alkyl *N*-[bis(dimethylamino)-methylidene]-*P*-methylphosphonamidate Novichok derivatives.

was stirred at 0–5 °C for 30 min. Compound **3** in DCM (200 µL) was then added dropwise to the solution, while stirring at 0–5 °C for 2 h. Any precipitate was filtered off and the resulting solutions of the end product were analyzed by GC/MS. It should be noted that, due to the extreme toxicity of these materials, the separation and purification of CWC-related chemical are very difficult and therefore should be carried out only by a trained professional in an efficient fume cupboard equipped with an active charcoal filtration system.

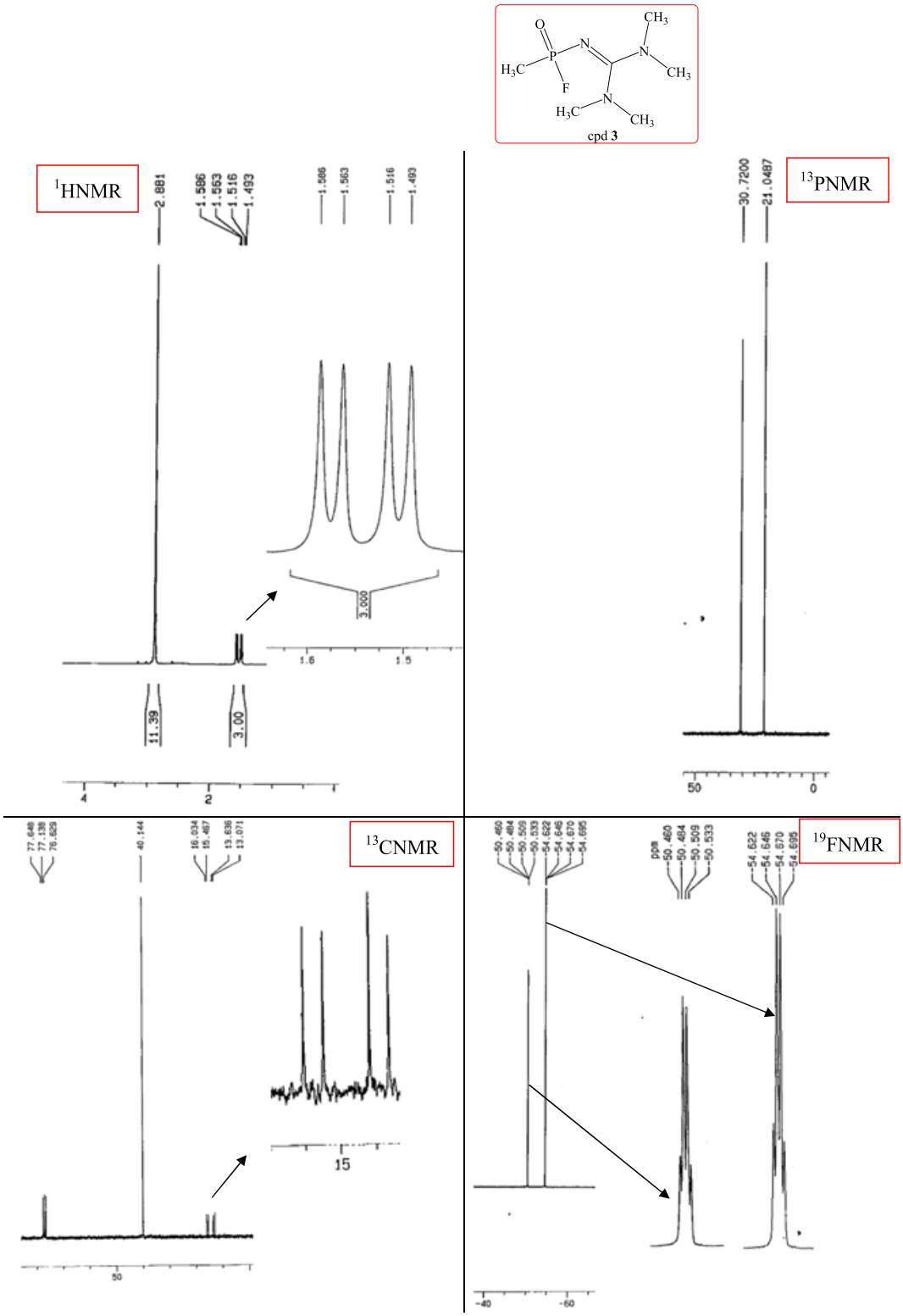


Figure 1. ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR spectra of cpd **3**.

RESULTS AND DISCUSSION

The microsynthesis of the *O*-alkyl *N*-[bis(dimethylamino)-methylidene]-*P*-methylphosphonamidates **4** generally involves two steps: the initial addition of *N,N,N′,N′*-tetramethylguanidine to methylphosphonic difluoride **1** solution in the presence of triethylamine as a base to form *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidic fluoride (**3**) and subsequently reaction with alcohols to yield the desired products **4**. It should be mentioned that cpd **3** is also covered under CWC schedule 2.B.04. Therefore, *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidic fluoride (**3**) was separated from the reaction mixture by column chromatography and its structure was fully characterized using ^{1}H, ^{13}C, ^{19}F and ^{31}P NMR and EI-MS analytical data (Fig. 1).

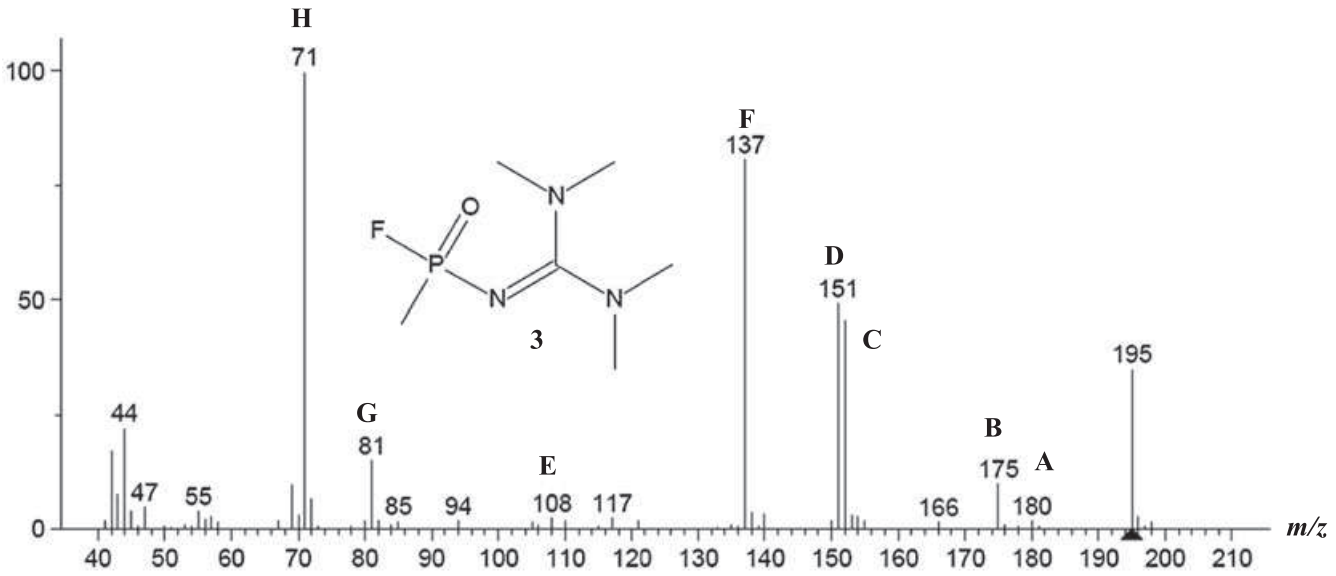
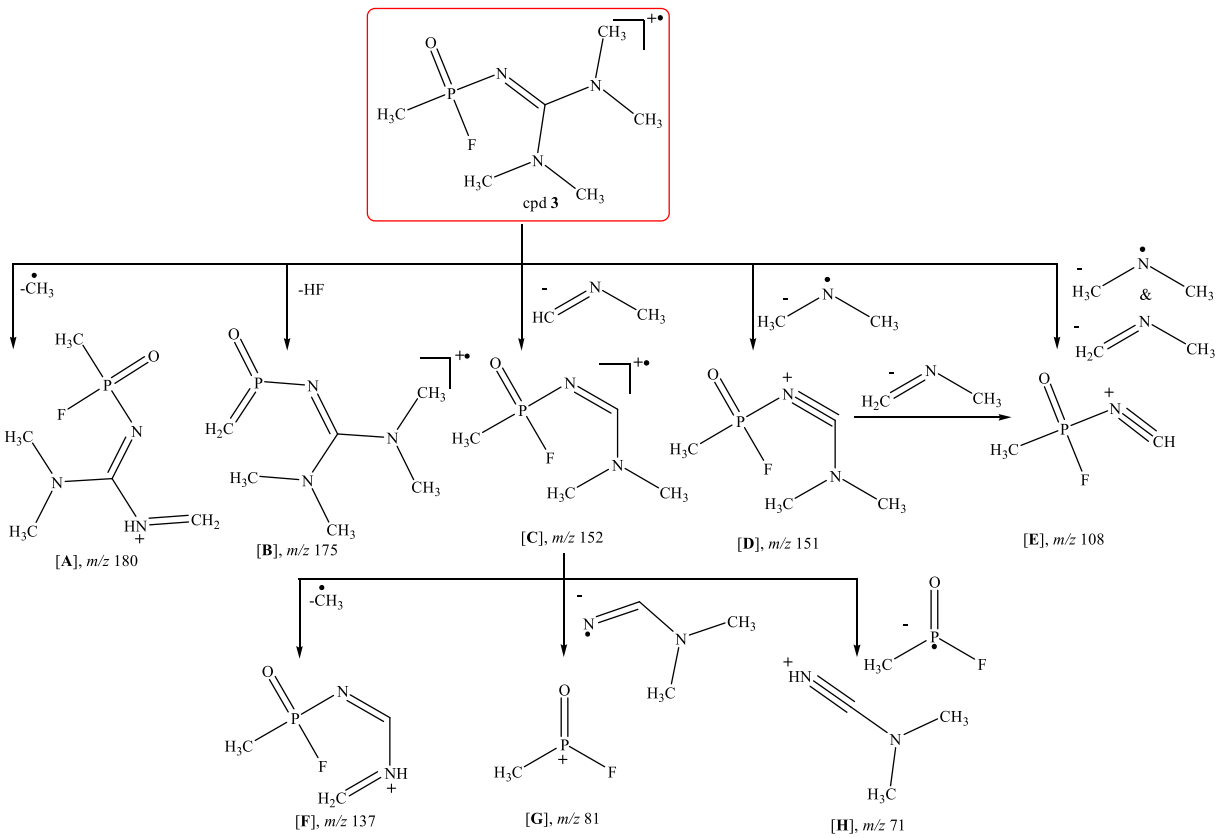


Figure 2. EI-MS spectrum of cpd **3**.



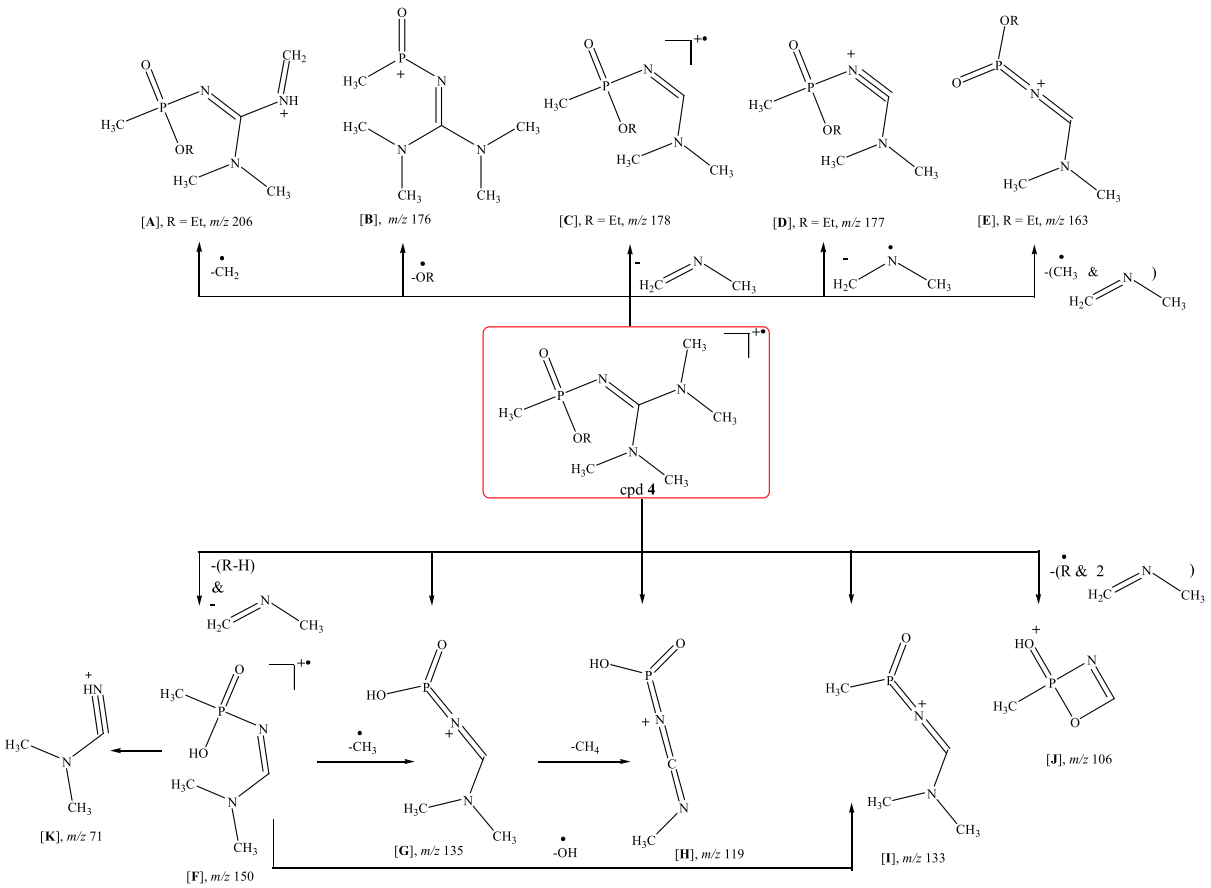
Scheme 2. General EI-MS fragmentation pattern of cpd **3**.

The ^{1}H NMR spectrum of **3** consisted of a doublet of doublet resonance ($^{2}J_{HP}$ = 17.5 Hz and $^{3}J_{HF}$ = 5.75 Hz) for the CH_3-P at δ = 1.49–1.58 ppm, and a singlet resonance (δ = 2.881 ppm) for the methyl protons on the nitrogen. The ^{1}H-decoupled ^{13}C NMR spectrum of cpd **3** showed two distinct resonances, in agreement with the proposed structure: at δ = 13.07–16.03 ppm a doublet of doublet resonance ($^{1}J_{CP}$ = 149.87 Hz and $^{2}J_{CF}$ = 35.37 Hz) for the carbon of CH_3-P and a singlet resonance at δ = 40.14 ppm for the carbon of the methyl protons on the nitrogen. The ^{31}P NMR spectrum of cpd **3** showed a distinct doublet resonance at δ = 21.04–30.72 ppm ($^{1}J_{PF}$ = 976.80 Hz). The ^{19}F NMR spectrum of cpd **3** showed a doublet of quartet resonance at δ = −54.69–(−50. 46) ppm ($^{1}J_{FP}$ = 978.07 Hz and $^{3}J_{FH}$ = 5.75 Hz). The EI mass spectrum of cpd **3** is shown in Fig. 2. The EI-MS spectrum of cpd **3** clearly showed the presence of the molecular ion ($M^{+\bullet}$) at *m/z* 195. Major EI fragment ions of cpd **3** are shown in Scheme 2. Direct elimination of a methyl radical on the nitrogen atom from $M^{+\bullet}$ resulted in the formation of ion [A]. Loss of HF from $M^{+\bullet}$ gave rise to ion [B], although with low relative abundance.

Fragment ion [C] is attributed to the loss of *N,N*-dimethylamine as *N*-methylidenemethanamine with a hydrogen rearrangement. Formation of the high intensity ion [D] from cpd **3** is through loss of a *N,N*-dimethylamino

Table 1. GC/MS data of *O*-alkyl *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidate Novichok derivatives

			Fragment ions (% relative abundances)										
Entry	R	$M^{+\bullet}$	[A]	[B]	[C]	[D]	[E]	[F]	[G]	[H]	[I]	[J]	[K]
1	CH_3	207(39)	192(1)	176(2)	164(68)	163(27)	149(100)	-	-	119(3)	133(11)	106(13)	71(26)
2	CD_3	210(42)	195(1)	176(1)	167(71)	166(30)	152(100)	-	-	120(2)	133(4)	106(9)	72(6)
3	CH_3CH_2	221(54)	206(1)	176(6)	178(63)	177(20)	163(52)	150(47)	135(50)	119(22)	133(28)	106(23)	71(100)
4	CD_3CD_2	226(83)	211(2)	176(5)	183(99)	182(41)	168(100)	151(38)	136(64)	120(24)	133(10)	106(17)	72(95)
5	*i*-Pr	235(44)	220(1)	176(8)	192(32)	191(5)	177(5)	150(40)	135(73)	119(19)	133(13)	106(20)	71(100)
6	*i*-Pr (d_6)	241(51)	226(1)	176(8)	198(40)	197(10)	183(2)	151(31)	136(82)	120(2)	133(13)	106(18)	72(100)
7	Ph	269(8)	254(2)	176(3)	226(13)	225(100)	211(35)	-	135(8)	119(1)	133(23)	106(60)	-
8	Ph (d_2)	271(7)	256(2)	176(6)	228(26)	227(94)	213(34)	-	135(19)	-	133(38)	106(100)	-
9	2,6 CH_3-Ph	297(36)	282(3)	176(21)	254(4)	253(12)	239(15)	-	135(3)	119(2)	133(84)	106(100)	-



Scheme 3. General EI-MS fragmentation pattern of cpd **4**.

radical. Expulsion of two *N,N*-dimethylamino groups from $M^{+\bullet}$ resulted in the formation of fragment ion [E] at *m/z* 108. The formation of the characteristic ion [F] at *m/z* 137 as an iminium structure can be explained by loss of a methyl radical from [C]. Fragment ion [G] can be formed by direct elimination of the *N,N,N′,N′*-tetramethylguanidine group from cpd **3**. The intense fragment ion [H], which formed the base peak in the EI-MS spectrum of cpd **3**, is produced from the *N,N,N′,N′*-tetramethylguanidine group of cpd **3** or from ion [C] through some hydrogen rearrangements.

MS data of Novichok derivatives 4

With these encouraging results, attention was focused on the EI and ESI spectra of *O*-alkyl *N*-[bis(dimethylamino) methylidene]-*P*-methylphosphonamidate Novichok derivatives (Scheme 1, cpd **4**). Major EI fragment ions of cpd **4** are given in Table 1. Plausible fragmentation routes for these compounds are also illustrated in Scheme 3.

The mass spectra of cpd **4** are affected by their respective *O*-alkyl group. The $M^{+\bullet}$ ion of each *O*-alkyl *N*-[bis (dimethylamino)methylidene]-*P*-methylphosphonamidate **4** was observed in all EI-MS spectra with relatively good to moderate abundance. Fragment ion [A] formed through α-cleavage of the CH_3–N bond with relatively low intensity. Ion [B] resulted from loss of the alkoxy radical from $M^{+\bullet}$ and was observed in all the EI-MS spectra of cpd **4**. Direct expulsion of the *N,N*-dimethylamino group as *N*-methylidenemethanamine with a hydrogen rearrangement gave rise to fragment ion [C] with relatively good abundance – in the range of 4–99 %. Corresponding peaks in the mass

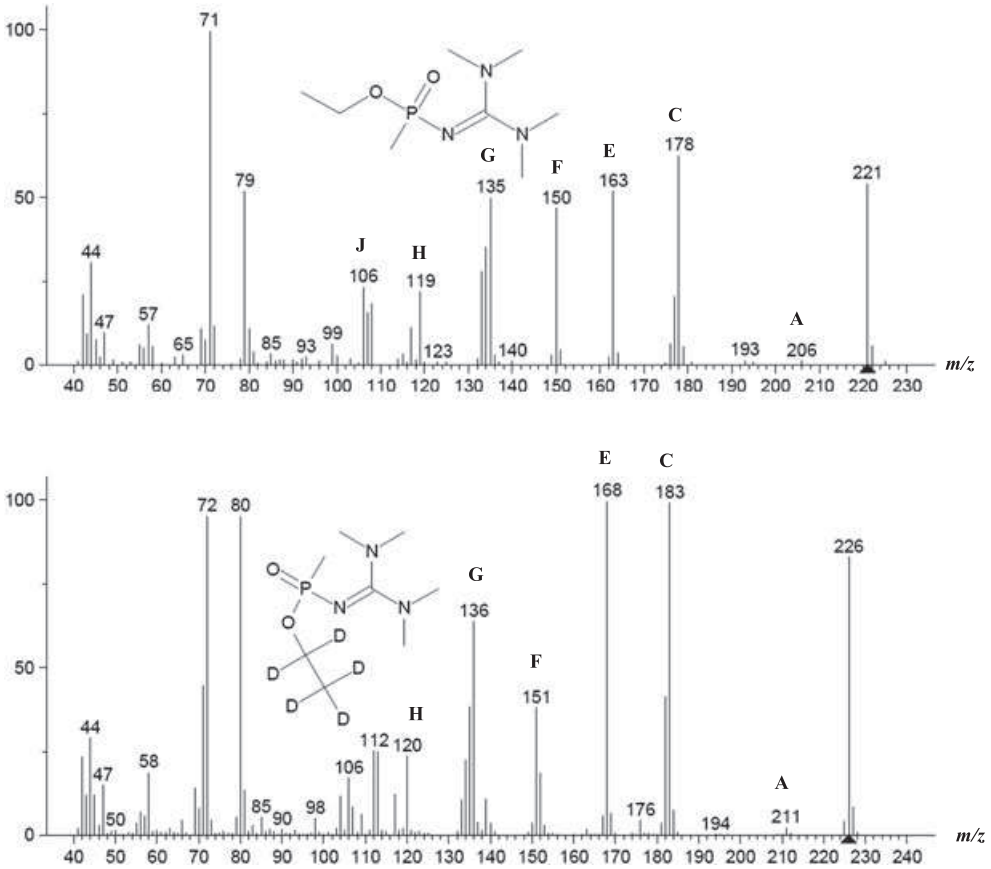
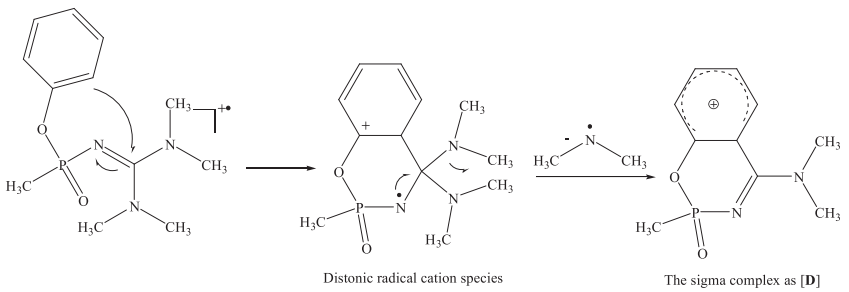


Figure 3. Representative EI-MS mass spectra of an *O*-ethyl Novichok derivative (top) and its deuterated analog (bottom).



Scheme 4. Expulsion of *N,N*-dimethylamino radical from *O*-aryl derivatives of cpd **4** by an intramolecular electrophilic aromatic substitution reaction.

spectra from deuterated analogs also supported this interpretation (Fig. 3). EI-MS spectra of all cpds **4** contained the ion [D], which corresponds to an iminium species. This ion could be generated from $M^{+\bullet}$ by direct elimination of *N,N*-dimethylamine. This fragment ion was the base peak or the second most abundant peak in the EI-MS spectra of compounds bearing phenyl on an oxygen atom (Table 1, entries 7 and 8). In the case of the phenyl derivatives, ion [D] (Scheme 3) can be produced *via* an intramolecular electrophilic aromatic substitution reaction, resulting in a distonic species. Subsequently, expulsion of *N,N*-dimethylamine led to fragment ion [D] (Scheme 4). The spectrum of the deuterated phenyl analog (Table 1, entry 8) clearly shows the ion corresponding to this fragment. Generation of a stable cation fragment (the sigma complex)[20] is the major driving force for this process. It

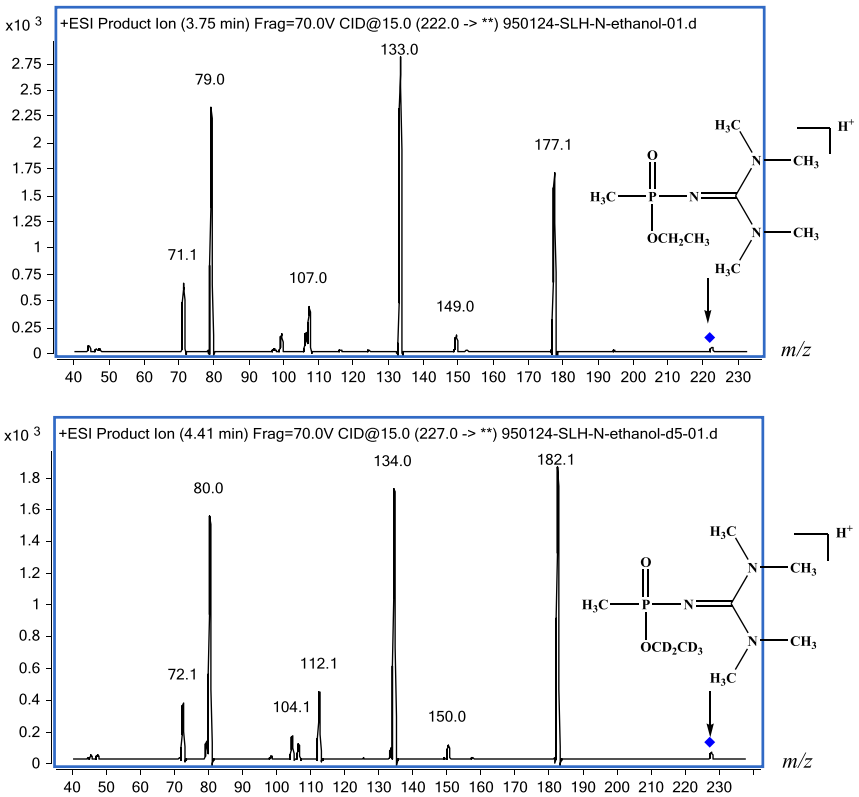
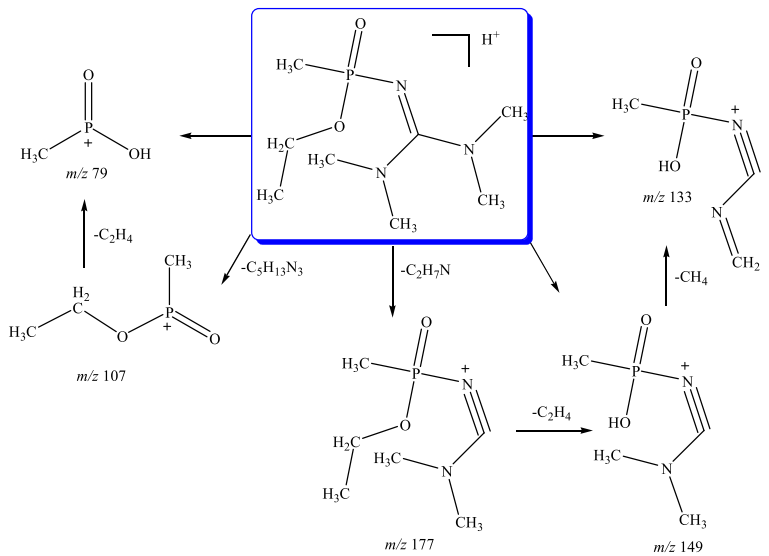


Figure 4. Product ion mass spectra of the protonated *O*-ethyl derivative of cpd **4** (top) and its deuterium-labeled analog ($-OC_2D_5$) (bottom).



Scheme 5. General collision-induced dissociation (CID) fragmentation pathways of the protonated *O*-ethyl derivative of **4**.

is noteworthy that in the case of *m*-xylene on oxygen, incorporation of the π-system for the expulsion of the *N*,*N*-dimethylamine radical is much lower than for the phenyl group (12 % vs 100 % intensity of ion [D]).

Concerted or step-wise expulsion of *N*-methylidene-methanamine and a methyl radical from $M^{+\bullet}$ resulted in a fragment (ion [E], Scheme 3) which is the base peak in the EI-MS spectra of the compounds bearing aminomethyl and ethyl moiety (entries 1, 2 and 3). The elimination of an alkene via McLafferty-type fragmentation then loss of *N*-methylidenemethanamine in mass spectra of cpd **4** led to ion [F] (Scheme 3). In the McLafferty-type rearrangement, the alkyl on the oxygen is left as an alkene. For the *O*-methyl and *O*-phenyl analogs formation of an allylic or vinylic group is not possible. Consequently, the spectra for these chemicals are distinct. Formation of ions [G], [H], [I], [J] and [K] can be explained by some step-wise processes and hydrogen rearrangements involving loss of methyl and hydroxyl radicals and methane, *N*-methylidenemethanamine and alkene from $M^{+\bullet}$ or fragment ion [F].

The ESI-MS/MS spectrum of the $[M+H]^+$ ion recorded on a quadrupole ion trap mass spectrometer for the *O*-ethyl derivative of the Novichok derivative (Scheme 1) is shown in Fig. 4. The proposed general fragmentation pathways of the protonated molecule shown in Scheme 5 are supported by the study of the ESI-MS/MS spectrum of the deuterated analog (Fig. 4). The ESI fragmentation pattern is similar to the EI fragmentation pattern.

Fragmentation of the *O*-ethyl derivative of cpd **4** produced an ion at *m/z* 177 with a relatively good abundance due to the loss of *N*,*N*-dimethylamine. The ions corresponding to the *O*-ethyl *m/z* 177 species in the product ion mass spectra of the protonated species of *O*-methyl, *O*-methyl (d_3) and *O*-phenyl derivatives of **4** (entries 1, 2 and 7) were also observed at *m/z* 163, 166 and 225, respectively. These *m/z* 177 ions further fragmented producing an ion at *m/z* 149 resulting from expulsion of C_2H_4. Expulsion of methane from the *m/z* 149 ion produced the most abundant product ion at *m/z* 133. Direct elimination of *N*,*N*,*N*′,*N*′-tetramethylguanidine from the $[M+H]^+$ ion led to the formation of a product ion at *m/z* 107 with a moderate abundance. Subsequent elimination of ethylene gave an ion at *m/z* 79. The proposed structural assignments for all the ions in Scheme 5 are supported by the deuterium-labeling studies, which show the formation of the corresponding ions in the product ion mass spectra of the protonated species of the deuterium-labeled analog ($-OC_2D_5$). It is interesting to note that CID fragmentation of the protonated *O*-phenyl derivative of cpd **4** shows a characteristic product ion at *m/z* 180 which resulted from elimination of two dimethylamines from $[M+H]^+$ (Fig. 5).

The ions corresponding to this fragmentation did not appear in the CID spectra obtained from the other Novichok derivatives of cpd **4**. The proposed pathway for the formation of this ion in the CID spectrum of the *O*-phenyl derivative of cpd **4** is shown in Scheme 6. The first step in this mechanism is an intramolecular electrophilic aromatic substitution reaction and expulsion of a dimethylamine molecule from $[M+H]^+$. This reaction produces a sigma complex, which then yields the product ion at *m/z* 180, via loss of another dimethylamine molecule.

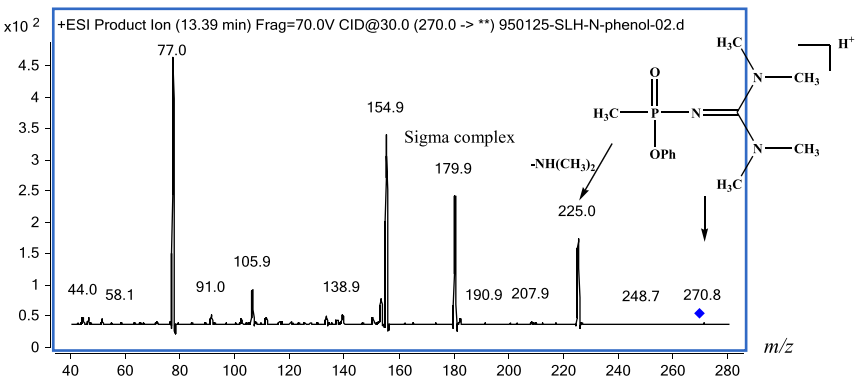
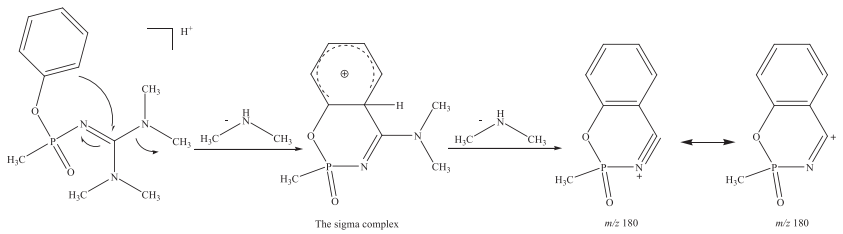


Figure 5. Product ion mass spectrum of the protonated *O*-phenyl derivative of cpd **4**.



Scheme 6. Proposed fragmentation pathway for the formation of ion at *m/z* 180 in the CID spectrum of the protonated *O*-phenyl derivative of cpd **4**.

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CONCLUSIONS

For the first time, EI-MS and ESI-MS/MS spectra of a series of *O*-alkyl *N*-[bis(dimethylamino)methylidene]-*P*-methylphosphonamidates **4** related to CWC were collected and investigated with the aim of enriching the Organization for the Prohibition of Chemical Weapons Central Analytical Database (OCAD), which may be used in OPCW verification activities, on/off site analysis, and to improve MS interpretation knowledge. The proposed mechanisms for the formation of the fragments were confirmed through the analysis of mass spectra of deuterated analogs.

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