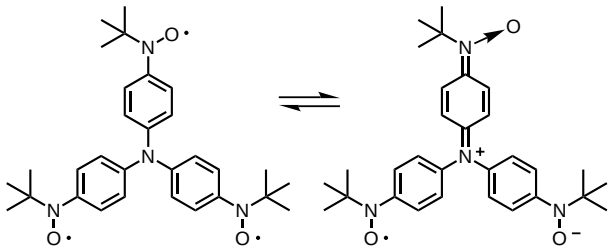

Scheme 1. Equilibrium of the triradical and zwiterionic structures of **2**.

Experimental Section

Tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine: To a solution of tris(*p*-bromophenyl)amine (2.0 g, 4.15 mmol) in THF (40 mL) was added *tert*-butyllithium (1.6 M pentane solution, 18.2 mL) at −78 °C. The mixture was warmed to 0 °C over 10 min and then cooled again to −78 °C. 2,2-Dimethylnitrosoethane (1.45 g, 16.6 mmol) was added, and the mixture was warmed to room temperature and stirred for 1 h. Saturated aqueous ammonium chloride and ether were added, and the organic layer was separated, washed with water, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was washed with dichloromethane to afford tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine (0.77 g, 37 %) as a white powder. M.p. 153 °C (decomp.); ^{1}H NMR (270 MHz, [D$_6$]DMSO): $\delta = 8.20$ (s, 3H), 7.92 (d, $J = 8.57$ Hz, 6H), 6.84 (d, $J = 8.57$ Hz, 6H), 1.06 (s, 27H); ^{13}C NMR (67.8 MHz, [D$_6$]DMSO): $\delta = 145.34$, 143.74, 125.29, 122.23, 59.19, 25.99; FAB MS calcd for $C_{30}H_{42}N_4O_3$: 506.3257, found: 506.3256.

2: To a solution of tris[*p*-(*N*-*tert*-butyl-*N*-hydroxyamino)phenyl]amine (100 mg, 0.20 mmol) in dichloromethane (30 mL) was added an excess of freshly prepared Ag_2O (ca. 300 mg), and the mixture was stirred for 2 h. The reaction mixture was filtered, and the solvent was removed under reduced pressure at ambient temperature. The residue was subjected to chromatography on aluminum oxide with dichloromethane as eluent and recrystallized from *n*-heptane/dichloromethane (2/1) to give **2** as dark violet crystals (86 mg, 87 %). M.p. 190–192 °C; FAB MS: *m*/*z* 503 [*M*$^+$]; elemental analysis calcd for $C_{30}H_{39}N_4O_3$: C 71.54, H 7.80 N 11.12; found: C 71.44, H 7.79, N 11.08.

Magnetic measurement: Fine crystalline or polymer film samples were mounted in a capsule (Japan Pharmacopoeia NO. 5, ∅4.5 × 11 mm) and measured on a Quantum Design MPMS-5S SQUID susceptometer at 500 G. Corrections for the diamagnetic contribution were made with Pascal's constants.

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[4] Crystallographic data for **2**: $C_{30}H_{39}N_4O_3$, $M_r = 503.66$, monoclinic, space group *Cc*, $a = 13.11(1)$, $b = 23.081(5)$, $c = 10.410(2)$ Å, $\beta = 116.80(5)^\circ$, $V = 2810(2)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.190$ g cm^{-3}, $\mu(Mo_{K\alpha}) = 0.77$ cm^{-1}. Data were collected on a Rigaku RAXIS-IV imaging plate area detector at −100 °C with graphite-monochromated $Mo_{K\alpha}$ radiation ($\lambda = 0.71071$ Å). The structure was solved with direct methods and refined to $R = 0.039$, $R_W = 0.054$ for 2461 observed reflections ($I > 3.00\sigma(I)$) and 335 parameters. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-101263. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

[5] Symmetry operation: $x, -y, 1/2 + z$.

[6] The origin of the ferromagnetic interaction between the neighboring molelucles in crystals may be located at O1−C12′ and O3−C25′ (3.272(4) and 3.131(3) Å, respectively) along the chain made by the molecules aligned in the direction of the *c* axis.

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[8] Triradical **2** exhibits a characteristic broad band between 450–800 nm ($\lambda_{max} = 534$ nm, $\varepsilon = 5500$ M^{-1} cm^{-1}), but its absorption coefficient is relatively weak compared with that of quinonoid system **1** ($\lambda_{max} = 554$ nm, $\varepsilon = 20400$ M^{-1} cm^{-1}).[2] The X-ray crystal structure shows that the *p*-phenylene rings have a slight tendency to assume a quinonoid structure, and there are signs of shortened bond lengths in the bonds connecting a aminoxyl nitrogen atoms with the benzene rings (1.43–1.45 Å is typical of phenylaminoxyl groups).[9, 10] However, compared with the carbon analogue **1**, where two *p*-phenylene rings exhibit quinonoidal bond alternations of 0.05 Å, any bond alternation is less than 0.02 Å in **2**. The dihedral angles between the benzene rings and the central trigonal nitrogen plane are somewhat larger for a quinonoid structure.

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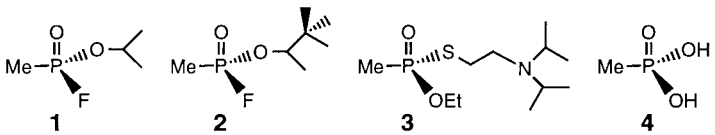
Monitoring Chemical Warfare Agents: A New Method for the Detection of Methylphosphonic Acid**

Jon A. Ashley, Chao-Hsiung Lin, Peter Wirsching,* and Kim D. Janda*

Issues surrounding chemical warfare agents are currently of great importance with regard to national security and world affairs. The lethal compounds **1** (sarin), **2** (soman), and **3** (VX)



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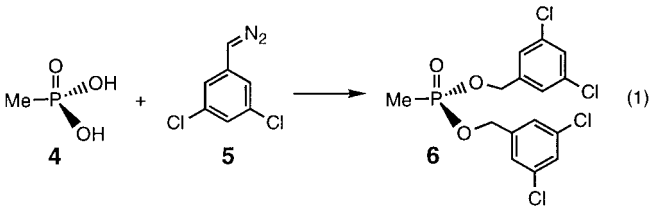
have been feared as the "nuclear weapons" of the poorer nations because their manufacture is relatively simple and the starting materials are inexpensive and readily available. Recently, sarin was implicated in attacks by Iraq on Kurdish communities[1] and in the terrorist activity in the Tokyo subway.[2] Since these and other nerve agents degrade naturally in the environment to methylphosphonic acid (MPA) **4** or can be degraded in the laboratory to MPA, a convenient detection method for this substance is necessary. Therefore, the development of a simple, portable, and inexpensive immunoassay kit would be valuable to monitor various treaty compliances and also during military operations.[8]

As a result of immunogenicity problems, monoclonal antibodies (mAbs) that bind MPA could not be successfully obtained. Consequently, we reasoned that if MPA were readily derivatized with recognition elements, mAbs that bound the MPA derivative could be elicited by using a structurally congruent, immunogenic hapten. In this way, the presence of MPA itself could be assessed indirectly through formation and detection of the derivative. Since diazomethane rapidly and quantitatively forms methyl esters from either carboxylic or phosphonic acids,[3] it was anticipated that a more complex diazo compound might also prove useful. We decided to utilize 3,5-dichlorophenyldiazomethane **5** since this compound was prepared in high yield and showed excellent stability.[4] Moreover, the dichloro-substituted aromatic ring presented a potent epitope as part of the hapten structure.

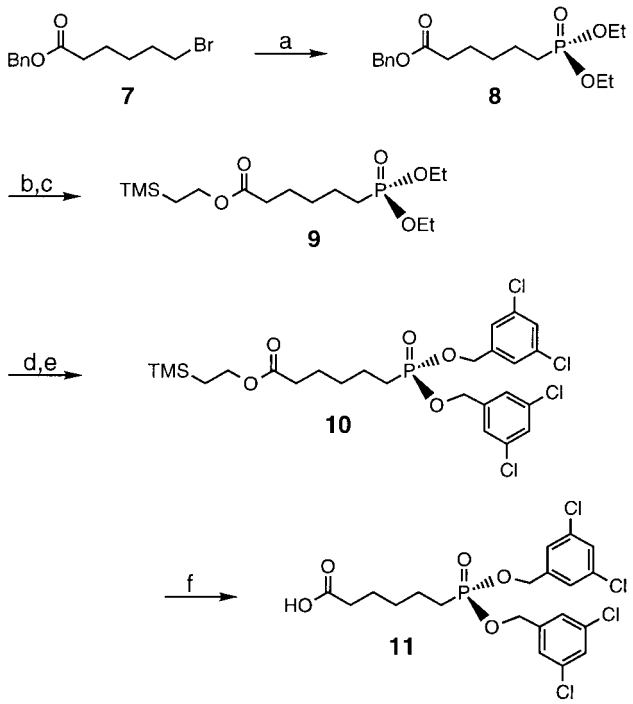
The best solvent for the esterification of **4** with **5** [Eq. (1)] was found to be dioxane/0.5% water wherein the small amount of water was used to ensure complete solubility of



(1)

MPA. Although the yield of isolated and purified bis(3,5-dichlorobenzyl) methylphosphonate **6** was good (67%), the estimated in situ yield was near 80% by visual colorimetric comparison to authentic standard solutions eluted by thin-layer chromatography and stained with cerium molybdate. The remaining 20% of the mass balance was accounted for by an impurity in **5** (10%) and the formation of a by-product, MPA mono(3,5-dichlorobenzyl) ester (10%). The latter was formed since we chose to use only a stoichiometric amount of **5** (2 equiv). Despite the presence of water in the reaction, hydrolysis of the diazo reagent to 3,5-dichlorobenzyl alcohol was not observed.

The hapten **11** (CDC) corresponding to the derivative **6** was then prepared (Scheme 1). The CDC was conjugated to the carrier protein keyhole limpet hemocyanin (KLH) and entered into our standard immunization program in mice. An enzyme-linked immunosorbent assay (ELISA) protocol in a competition format[5] was used during the hybridoma



Scheme 1. Synthesis of the hapten **11** structurally related to the MPA derivative **6**. a) diethyl phosphite (1.2 equiv), NaH (1.2 equiv), DMF, 0 °C→25 °C, 2 h; b) H_2, Pd/C, methanol, 25 °C, 1 h; c) 2-trimethylsilylethanol (1.2 equiv), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC, 1.2 equiv), 4-dimethylaminophenol (DMAP, cat), DMF, 25 °C, 16 h, d) 1) Me_3SiBr (2.1 equiv), CH_2Cl_2, 25 °C, 1 h; 2) oxalyl chloride (3 equiv), DMF (cat.), CH_2Cl_2, 25 °C, 1 h; e) 3,5-dichlorobenzyl alcohol (2.1 equiv), NEt_3 (2.5 equiv), DMAP (cat), 25 °C, 16 h; f) trifluoroacetic acid (TFA, 5 equiv), 0 °C→25 °C, 1 h.

screening and cloning process to increase the likelihood of cloning antibodies with high specificity for **6**. Hence, clones were selected on the basis of the best binding of the free ligand **6** versus a bovine serum albumin (BSA) conjugate of **11**. A panel of 11 mAbs that bound **6** were isolated of which CDC27B4 showed the highest affinity ($K_d \approx 1\,\mu M$). Based on previous work in our laboratory, this apparent K_d was likely up to an order of magnitude higher than the true K_d since competition ELISA tends to overestimate the value. Importantly, CDC27B4 showed excellent specificity when examined for cross-reactivity versus alternate ligands **4**, and **12**–**18** .

At concentrations of 10 μM, no binding of these compounds was detected using competition ELISA. Even at 100 μM of **16**, the derivative of the first breakdown product of sarin, no binding was observed. Given the presence of the 3,5-dichlorophenyl substituent as an anticipated strong haptenic determinant, the specificity was rather exquisite. The results suggested that both rings were recognized during the immune response and were required for binding. Moreover, the antibody was specific for the chemical nature and coordination sphere of the phosphorus species as revealed by the phosphate esters **17** and **18**. Despite the presence of at least two 3,5-dichlorophenyl substituents, the hydroxyl group of **17** and the steric bulk of the extra ring of **18** precluded binding at 10 times the K_d of the derivative **6**. The K_d for these compounds was about 300 μM.

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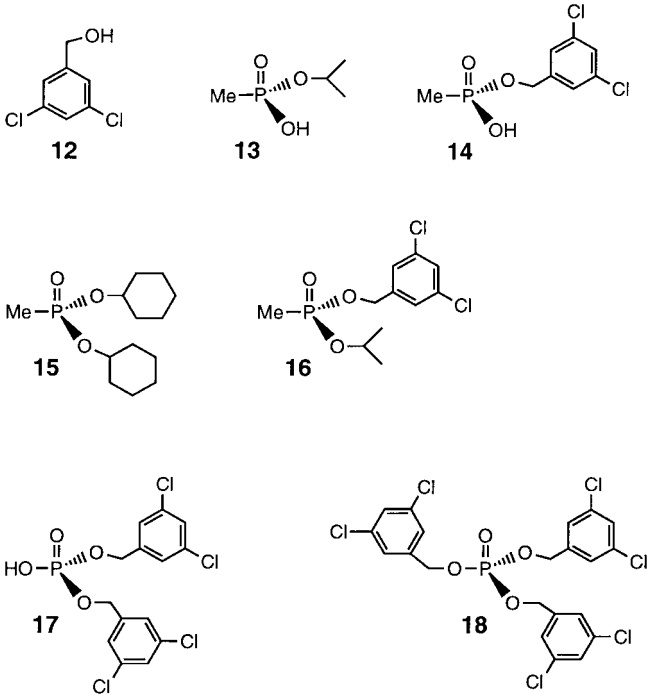

Based on principles of antibody fine specificity and that the ligands tested were structurally related to **6**, there should be no interference from other compounds present at test sites. It was estimated that 170 ± 10 ppb of MPA analyte could be assayed as derivative **6**. A sensitivity comparable to many reported instrumental technologies that are expensive and usually necessitate specialized training. The sensitivity was also similar to other immunoassays developed for chemical warfare agents themselves.[6] Depending on the confidence limit chosen for detection, we anticipate the derivatization–ELISA procedure will accurately reflect the presence of MPA in an appropriate field sample.

Other methods routinely used for MPA analysis invoked chromatographic and/or spectrometric techniques.[7] Most of these approaches also required a prior derivatization step and furthermore were generally not amenable for the testing of crude samples. Our method is inexpensive, sensitive, convenient, robust, and would require only a minimum of sample preparation. For instance, a field sample from a suspected chemical warfare agent manufacturing, storage, or deployment site could be obtained by swipe and extracted with dioxane/water or a collected wet sample evaporated and redissolved. A simple filtration step might then be employed followed by addition of the reagent **5** and then assay. Notably, the presence of **5** in a 100-fold molar excess relative to antibody had no detrimental effects on binding. Hence, no other handling or purification steps should be necessary. While refinements are necessary to establish a field kit, we believe derivatization–ELISA can complement other MPA detection methods. These efforts and adaptation to other compounds related to chemical warfare agents are in progress.

Experimental Section

6: Reagent **5** (187 mg, 1 mmol) was added slowly to a solution of MPA (48 mg, 0.50 mmol) in 1,4-dioxane/0.5 % water (5 mL). Bubbling was immediately observed upon addition. The resulting solution was stirred at room temperature for 1 h and then diluted with ethyl acetate (10 mL). After washing with brine (5 mL), the organic layer was dried with $MgSO_4$ and concentrated to give a yellow solid. The solid was purified using flash chromatography (95/5 CH_2Cl_2/EtOAc) affording a white solid (138 mg, 67 %).

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$[Mn(en)]_3[Cr(CN)_6]_2 \cdot 4H_2O$: A Three-Dimensional Dimetallic Ferrimagnet ($T_c = 69$ K) with a Defective Cubane Unit**

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Recently, there has been increasing interest in metal assemblies of ordered networks.[1–11] One fascinating target in this research area are molecular-based magnets that exhibit

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