Synthesis of Bis-pyridinium Oxime Antidotes Using Bis(methylsulfonoxymethyl) Ether for Organophosphate Nerve Agents

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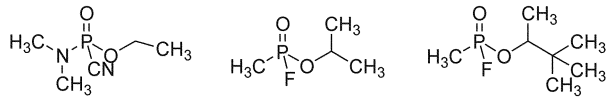
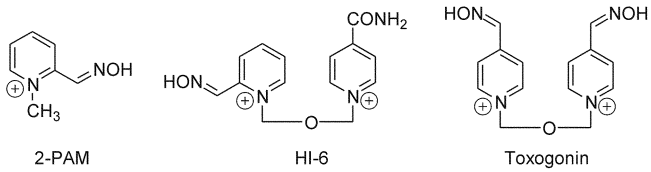
Organophospate nerve agents are organic esters of phosphorous-based acid derivatives and they are extremely toxic chemicals that were first developed before and during World War II primarily for military use. Five organophosphates are generally regarded as nerve agents. They are commonly known as dimethylphosphoramidocyanidic acid ethyl ester (tabun), methylphosphonofluoridic acid (1-methylethyl) ester (sarin), methylphosphonofluoridic acid 1,2,2-trimethylpropyl ester (soman), methylphosphonofluoridic acid cyclohexyl ester (cyclosarin), and methylphosphonothioic acid S-[2-[bis(1-methylethyl)amino] ethyl] O-ethyl ester (VX). Nerve agents are compounds that exert their biological effects by inhibition of the enzyme acetylcholinesterase (AChE), found at the receptor sites of tissue innervated by the cholinergic nervous system, which hydrolyzes acetylcholine (ACh) very rapidly.[1] The acute toxicity of organophospate compounds in mammals is generally believed to be due to their irreversible inhibition of AChE. Among these organophospate nerve agents, soman is probably one of the most dangerous organophospate agents since its deleterious effects are especially difficult to counteract.[2] Soman seems to cause centrally mediated seizure activity that can rapidly contribute to profound brain damage.



Tabun Sarin Soman

Current medical protection against the toxicity of organophospate compounds consists of a regimen of anticholinergic drugs, such as atropine, to counteract the accumulation of acetylcholine and oximes to reactivate organophospate-inhibited AChE.[3] It is clearly important that acetylcholinesterase reactivators should be available as antidotes of poisoning by such compounds. One of the most widely used is pralidoxime (2-PAM). Unfortunately, the presently used antidote, such as pralidoxime in combination with atropine, does not appear to ameliorate soman-induced toxic signs. Some bis-pyridinium oximes are known to be acetylcholinesterase reactivators and effective antidotes in intoxication with organophosphates.[4] The most important standard oximes are toxogonin, HI-6 and HGG-12. Although pyridinium oximes like 2-PAM can not reactivate soman-inhibited AChE, bis-pyridium oximes like HI-6 have been found to be effective reactivators of non-aged soman-inhibited human AChE.

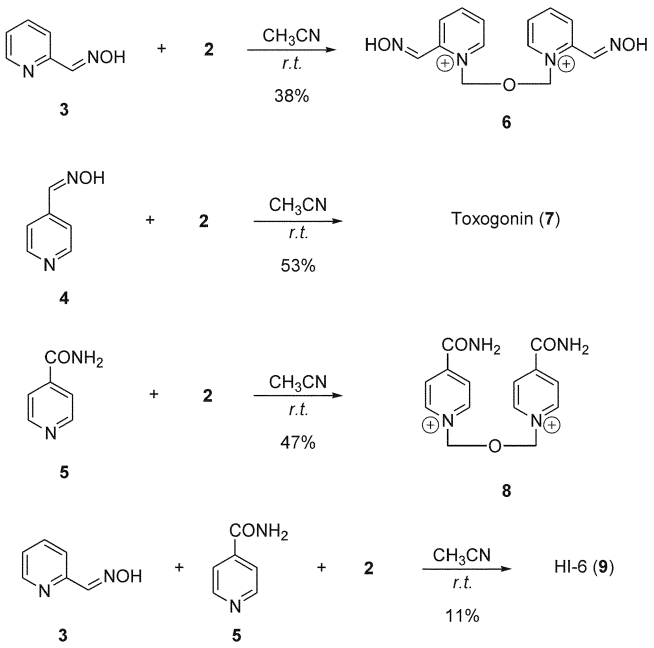


2-PAM HI-6 Toxogonin

In their manufacture, the bis-pyridinium oximes require the use of bis(chloromethyl) ether (BCME).[5] Recently, however, BCME has been found to be such a potent carcinogen that it is no longer commercially available. For safe handling, human beings simply must not be exposed to this substance in any concentration, no matter how slight it may be. Consequently, there is a need for a less volatile reagent for use in manufacturing nerve agent antidotes. There is a further need to replace BCME in nerve agent antidote production with a non-carcinogenic material. Bis(methylsulfonoxymethyl) ether,[6] which is non-carcinogenic, is substituted for the BCME in the production of bis-pyridinium oximes.

1 **2**

Herein we report the synthesis of several bis-pyridinium oximes including the common nerve agent antidotes, toxogonin and HI-6, by use of bis(methylsulfonoxymethyl) ether **2**. The ether **2** was prepared by a reported method.[6] The bis-pyridinium oximes such as toxogonin and HI-6 were prepared from the reactions of bis(methylsulfonoxymethyl) ether with 2-pyridinealdoxime **3**, 4-pyridinealdoxime **4** and/or isonicotinamide **5** at *r.t.* in MeCN, and all reaction products were obtained as methanesulfonate salts (Scheme 1). *para*-Substituted pyridines **4** and **5** provided higher yields than *ortho*-substituted pyridine **3**. In these reactions, unreacted pyridines **3**, **4**, **5** were also obtained as their



Scheme 1

methanesulfonate salts, which were identified and easily separated from the mixture by washing with EtOH. For the preparation of HI-6, the ether **2** was reacted with pyridinaldoxime **3** followed by isonicotinamide **5**. Of the several possible products, bis-isonicotinamide **8** that is insoluble in MeOH was removed from the mixture by MeOH washing. The filtrate contained 1 : 1 mixture of **9** and a salt of **5**, and the both compounds were not separable in various solvent systems. For the identification, the 1 : 1 mixture was passed through a Dowex resin to give HCl salts of **9** and **5**, and pure HCl salt of **9** was prepared by use of BCME as a known method.[5] ^{1}H NMR spectrum of the HCl salt of **9** and ^{1}H NMR spectrum of the methanesulfonate salt of **9** were quite similar each other except the presence of a methyl peak in the methanesulfonate salt **9**.

Experimental Section

All reactions were carried out under N_2 atmosphere unless otherwise noted. MeCN was distilled from CaH_2 prior to use. Organic extracts or filtrates were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Flash chromatography was performed with Merck-EM Type 60 (230-400 mesh) silica gel (flash). ^{1}H NMR spectra were measured by Varian Gemini 200 MHz and Bruker AM-300 NMR spectrometers. Chemical shifts are reported in ppm (d) relative to TMS as internal standard. Mass spectrometric data determined by use of the electron impact (EIMS) method are reported as m/z (relative intensity). Elemental analyses were performed with CE Instruments-ea 1110 Automatic Elemental Analyzer. Melting points were uncorrected.

1,1'-[Oxybis(methylene)]-bis[2-(hydroxyimino)methyl]-pyridinium dimethanesulfonate (6). To a solution of 2-Pyridinealdoxime (3.91 g, 31.9 mmol) in CH_3CN (20 mL) was added a mixture of bis(methanesulfonoxymethyl)ether (**2**) (5.0 g, 21.3 mmol) in CH_3CN (3.7 mL) by use of cannular needle at 0 °C. The mixture was stirred at 0 °C for 0.5 h followed by at *r.t.* for 20 h. A precipitate was washed with EtOH (12.5 mL) and dried *in vacuo* to give **6** (2.90 g, 38%). Analytical sample was obtained by recrystallization from MeOH. ^{1}H NMR (300 MHz, D_2O) δ 2.60 (s, 6H, $2CH_3SO_3$), 6.24 (s, 4H, $2CH_2O$), 7.87-7.92 (m, 2H, ArH), 8.28 (d, J = 8.0 Hz, 2H, 2CHN), 8.43-8.48 (m, 4H, ArH), 8.79 (d, J = 6.1 Hz, 2H, Ar); ^{13}C NMR (75 MHz, D_2O) 40.1, 87.2, 128.9, 129.5, 143.1, 146.5, 148.5, 149.7; Mass (FAB) m/e (rel. intensity) 479 (3), 383 (48), 307 (7), 287 (100), 257 (31), 239 (10), 165 (100), 119 (100); mp 167-170 °C.

1,1'-[Oxybis(methylene)]-bis[4-(hydroxyimino)methyl]-pyridinium dimethanesulfonate (7). To a solution of 4-Pyridinealdoxime (0.33 g, 2.69 mmol) in CH_3CN (9 mL) was added a mixture of bis(methanesulfonoxymethyl)ether (**2**) (0.42 g, 1.79 mmol) in CH_3CN (1 mL) by use of cannular needle at 0 °C. The mixture was stirred at 0 °C for 0.5 h followed by at *r.t.* for 20 h. A precipitate was washed with EtOH (5 mL) and dried *in vacuo* to give **7** (2.9 g, 38%). Analytical sample was obtained by recrystallization from MeOH. ^{1}H NMR (200 MHz, D_2O) δ 2.64 (s, 6H, $2CH_3SO_3$), 6.05 (s, 4H, $2CH_2O$), 8.14 (d, J = 7.3Hz, 4H, ArH), 8.25 (s, 2H, 2CHN), 8.84 (d, J = 6.5Hz, 4H, ArH); ^{13}C NMR (75 MHz, D_2O) 38.8, 87.1, 125.3, 144.0, 146.4, 151.7; Mass (FAB) m/e (rel. intensity) 479 (7), 383 (100), 287 (25), 261 (11), 231 (5), 142 (22), 112 (5); mp 210-211 °C.

1,1'-[Oxybis(methylene)]-bis[4-carbamoyl]pyridinium dimethanesulfonate (8). To a solution of isonicotinamide (5.62 g, 46 mmol) in CH_3CN (20 mL) was added a mixture of bis(methanesulfonoxymethyl)ether (**2**) (7.2 g, 30.7 mmol) in CH_3CN (3.7 mL) by use of cannular needle at 0 °C. The mixture was stirred at 0 °C for 0.5 h followed by at *r.t.* for 20 h. A precipitate was washed with EtOH (12.5 mL) and dried *in vacuo* to give **8** (5.2 g, 47%). ^{1}H NMR (300 MHz, D_2O) δ 2.60 (s, 6H, $2CH_3SO_3$), 6.16 (s, 4H, $2CH_2O$), 8.32 (d, J = 6.8 Hz, 4H, ArH), 9.08 (d, J = 6.8 Hz, 4H, ArH); ^{13}C NMR (75 MHz, D_2O) 40.1, 88.4, 127.9, 146.0, 152.1, 167.1; Mass (FAB) m/e (rel. intensity) 479 (11), 460 (6), 383 (100), 365 (5), 307 (55), 288 (82), 261 (69), 231 (16), 154 (100), 106 (77); mp 205-206 °C.

1-(2-Hydroxyiminomethyl-1-pyridino)-3-(4-carbamoyl-1-pyridino)-2-oxapropane dimethanesulfonate (9). To a solution of 2-pyridinealdoxime (0.32 g, 2.5 mmol) in CH_3CN (9 mL) was added a mixture of bis(methanesulfonoxymethyl)ether (**2**) (0.60 g, 2.5 mmol) in CH_3CN (1 mL) by use of double tip needle at 0 °C. The mixture was stirred at 0 °C for 0.5 h followed by at *r.t.* for 3 h. A solution of isonicotinamide (0.32 g, 2.5 mmol) in CH_3CN (9 mL) was slowly added at 0 °C. After stirring at *r.t.* for 12 h, a precipitate was collected and washed with MeOH (12 mL) to give **8**. The filtrate was concentrated and the obtained solid was washed with EtOH (10 mL) and dried *in vacuo* to give a mixture (0.2 g) of **9** and **5**. The amount ratio of **9** and **5**

was 1 : 1, and **9** could be obtained 0.14 g (11%). ^{1}H NMR (200 MHz, D_2O) δ 2.62 (s, 6H, $2CH_3SO_3$), 6.12 (s, 2H, CH_2O), 6.25 (s, 2H, CH_2O), 7.92-7.99 (m, 1H, ArH), 8.29-8.35 (m, 3H, CHN and ArH), 8.44-8.55 (m, 2H, ArH), 8.88 (d, J = 6.0 Hz, 1H, ArH), 9.05 (d, J = 6.6 Hz, 2H, ArH).

[4-Carbamoyl]pyridium methanesulfonate (salt of 5). To a solution of isonicotinamide (1.00 g, 8.2 mmol) in CH_3CN (10 mL) was added a solution of methanesulfonic acid (0.54 mL, 8.2 mmol) in CH_3CN (1 mL) at 0 ^{o}C. After stirring at *r.t.* for 0.5 h. A precipitate was washed with CH_3CN and dried *in vacuo* to give a salt of **5** as a white solid (1.71 g, 96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.60 (s, 3H, CH_3SO_3), 8.15 (d, J = 6.8 Hz, 2H, ArH), 8.76 (d, J = 6.8 Hz, 2H, ArH); mp 199-202 ^{o}C.

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References

1. (a) Marrs, T. C. *Pharmacol. Ther.* **1993**, *58*, 51-66. (b) Dawson, R. M. *J. Appl. Toxicol.* **1994**, *14*, 317-331.
2. Bajgar, J. *Acta Med.* **1996**, *39*, 101-105.
3. (a) Leadbeater, L.; Inns, R. H.; Rylands, J. M. *Fundam. Appl. Toxicol.* **1985**, *5*, S225-231.
4. (a) Thiermann, H.; Seidl, S.; Eyer, P. *Int. J. Pharm.* **1996**, *137*, 167-176. (b) Aas, P. *Eur. J. Pharmcol.* **1996**, *301*, 59-66. (c) Lallement, G.; Clarencon, D.; Brochier, G.; Baubichon, D.; Galonnier, M.; Blanchet, G.; Mestries, J. *Pharmacol. Biochem. Behav.* **1997**, *56*, 325-332.
5. Hagedorn, I. *USP 3,852,294*, 1974.
6. (a) Burness, D. M.; Wright, C. J.; Perkins, W. C. *J. Org. Chem.* **1977**, *42*, 2910-2913. (b) Wadsworth, D. H.; Vinal, R. S. *ibid* **1982**, *47*, 1623-1626.