Synthetical approaches to investigate protein-nerve agents adducts

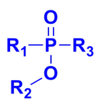
Introduction

Chemical warfare agents (CWAs) became well known with the World War I. Nowadays, the interest towards these compounds remains constant despite the majority of the states have ratified the Chemical Weapons Convention.[1] Nerve agents (OP) (Scheme 1), who are irreversible acetylcholinesterase inhibitors, are the most toxic. Unequivocal methods to verify exposure to them give credibility to the verification regime of the Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on their Destruction. Because of their stability and their specificity, adducts formed between nerve agents and proteins are good candidates as biomarkers for the analytical investigation.[2]

Furthermore, these adducts can give important indications about the nerve agent's actions in the body.

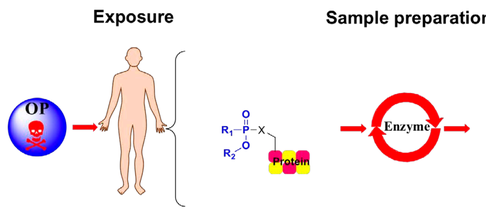
Project

Nerve agents



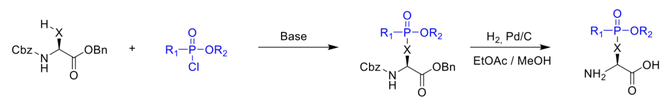
G-Agents Tabun (GA), $R_1 = R_2$ =Et, R_3 = CN
Sarin (GB), R_1 = Me, R_2 = iPr, R_3 = F
Soman (GD), R_1 = Me, R_2 = Pinacolyl, R_3 = F
Cyclosarin (GF), R_1 = Me, R_2 = Cy, R_3 = F

V-Agents VX, R_1 = Me, R_2 = Et, $R_3 = S(CH_2)_2N(iPr)_2$
R-VX, R_1 = Me, R_2 = iBu, $R_3 = S(CH_2)_2N(Et)_2$
C-VX, R_1 = Me, R_2 = nBu, $R_3 = S(CH_2)_2N(Et)_2$



Results

Amino acids bioadducts



Ser X = CH_2O, Tyr X = $CH_2C_6H_4O$, Thr X = $CH(CH_3)O$, Lys X = $(CH_2)_4NH$, Cys X = CH_2S[a], Trp X = $CH_2(C_8H_5N)$, Arg X = $(CH_2)_3NHC(NH)_2$

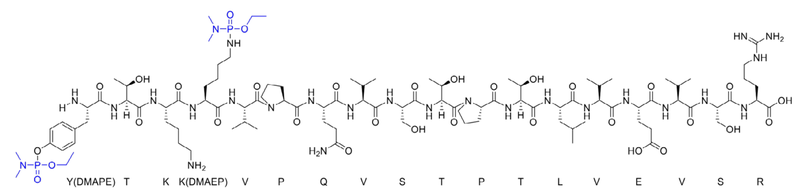
Entry	Methylphosphono-chloridate	Amino Acid	Product 1	Base	Time (min)	Yield (%)[b]	Product 2	Time (min)	Yield (%)[b]
1-7	Simi-VX	1 = Ser		DABCO	180	**93**		60	**61**
		2 = Tyr		DABCO	o. n.	**98**		45	**96**
		3 = Thr		DABCO	o. n.	**90**		30	**98**
		4 = Lys		Et_3N	60	**91**		120	**81**
		5 = Cys		DMAP	300	**75**		2 days	**68**
		6 = Trp		tBuOK	120	**35**		240	**77**
		7 = Arg		Et_3N	120	**34**		120	**98**
8-14	Simi-Sarin (GB)	8 = Ser		DABCO	o. n.	**57**		45	**96**
		9 = Tyr		DABCO	180	**79**		30	**89**
		10 = Thr		DABCO	2 days	**48**		60	**96**
		11 = Lys		Cs_2CO_3	60	**78**		240	**95**
		12 = Cys		DMAP	300	**89**		450	**88**
		13 = Trp		tBuOK	120	**49**		240	**93**
		14 = Arg		Et_3N	120	**53**		180	**73**
15-21	Simi-Soman (GD)	15 = Ser		Et_3N/DMAP	o. n.	**91**		30	**44**
		16 = Tyr		Et_3N/DMAP	210	**79**		60	**85**
		17 = Thr		Et_3N/DMAP	o. n.	**80**		45	**65**
		18 = Lys		Cs_2CO_3	60	**82**		150	**98**
		19 = Cys		DMAP	240	**72**		2 days	**65**
		20 = Trp		tBuOK	120	**34**		240	**83**
		21 =Arg		Et_3N	120	**34**		300	**51**
22-28	Simi-cyclosarin (GF)	22 = Ser		DABCO/DMAP	o. n.	**72**		60	**97**
		23 = Tyr		Et_3N/DMAP	240	**83**		60	**89**
		24= Thr		Et_3N/DMAP	o. n.	**88**		60	**87**
		25 = Lys		Cs_2CO_3	60	**71**		150	**92**
		26 = Cys		DMAP	240	**77**		o.n.	**76**
		27 = Trp		tBuOK	120	**33**		240	**85**
		28 = Arg		Et_3N	120	**40**		o.n.	**62**
29-35	Simi-chinese VX	29 = Ser		DABCO/DMAP	120	**90**		45	**98**
		30 = Tyr		DABCO/DMAP	o. n.	**82**		45	**99**
		31 = Thr		DABCO/DMAP	o. n.	**90**		30	**99**
		32 = Lys		Cs_2CO_3	60	**94**		135	**94**
		33 = Cys		DMAP	240	**72**		420	**81**
		34 = Trp		tBuOK	120	**44**		240	**59**
		35 = Arg		Et_3N	120	**27**		300	**82**
36-42	Simi- russian VX	36 = Ser		DABCO/DMAP	o. n.	**87**		30	**97**
		37 = Tyr		DABCO/DMAP	2 days	**77**		45	**96**
		38 = Thr		DABCO/DMAP	o. n.	**86**		30	**98**
		39 = Lys		Cs_2CO_3	60	**83**		120	**51**
		40 = Cys		DMAP	240	**76**		330	**83**
		41 = Trp		tBuOK	120	**7**		150	**54**
		42 = Arg		Et_3N	120	**27**		300	**82**
43-49	Simi-tabun (GA)	43 = Ser		DABCO/DMAP	480	**57**		60	**qualitative**[c]
		44 =Tyr		Et_3N/DMAP	o. n.	**92**		120	**99**
		45 = Thr		DABCO/DMAP	o. n.	**0**		n. a.	**n. a.**
		46 = Lys		Cs_2CO_3	60	**79**		120	**89**
		47 = Cys		DMAP	360	**61**		420	**9**
		48 = Trp		tBuOK	120	**36**		240	**81**
		49 = Arg		NaH	120	**22**		120	**92**

[a] Boc and t-buty protecting groups were used (hydrogenolysis step did not work with cysteine). Removal with TFA / DCM (1:1).
[b] Isolated yields.
[c] Product is not stable

Peptidic bioadducts

Protecting groups adaptation permits the solid phase synthesis of phosphylated peptides

Pepsin digest of human butyrylcholinesterase incubated with C-VX. [2] [3]



This amino acids sequence was observed after incubation of human serum albumine with DFP (diisopropylfluorophosphate) followed by trypsin digestion. [4]

Figure 1

Stability of sarin adducts in different media

Adduct	D_2O (without adjunction) pH 5-6, 25 °C	D_2O + HCl pH < 1, 25 °C	D_2O + NaOH pH > 14, 25 °C	Surine pH 7, 37 °C	Trizma buffer pH 7.4, 37 °C
H-Ser(MPIP)-OH					
H-Cys(MPIP)-OH					
H-Trp(MPIP)-OH					
H-Lys(MPIP)-OH					
H-Tyr(MPIP)-OH					
H-Thr(MPIP)-OH					
H-Arg(MPIP)-OH					

$t_{1/2} \geq 60$ days	$11 \leq t_{1/2} \leq 60$ days	$t_{1/2} \leq 10$ days

Table 2

Acknowledgement

This project has been developed in cooperation with the group of Prof. Bochet, Chemical Department of the University of Fribourg, Switzerland

References

[1] Convention on the Prohibition of the Development, Production, Stockpilling and Use of Chemical Weapons and on their Destruction, Organisation for the Prohibition of Chemical Weapons, The Hague 1994.

[2] Black, R. M.; Noort, D. In Chemical Warfare Agents: Toxicology and Treatment, 2nd ed.; John Wiley & Sons, L., Ed. 2007, p 127.

[3] MacDonald, M.; Lanier, M.; Cashman, J. Synlett 2010, 1951.

[4] Grigoryan, H.; Li, B.; Xue, W.; Grigoryan, M.; Schopfer, L. M.; Lockridge, O.Anal. Biochem. 2009, 394, 92.

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