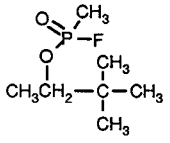
Stereoisomers of a Simulant of the Nerve Agent Soman. Synthesis and Protein Conjugation

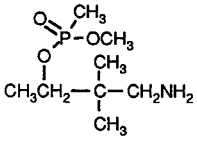
Robert M. Moriarty,* Anping Tao, Angelica Wendler, Om Prakash, Kaiming Liu, and Richard Gilardi
Department of Chemistry, Univeristy of Illinois at Chicago, Chicago, Illinois 60607, USA
Received 3 April 1997

Abstract: Four stereoisomers of a Soman simulant **2** were synthesized and conjugated to carrier proteins BSA and HSA for monoclonal antibody generation.

The nerve agent Soman, 3,3-dimethyl-2-butyl methylphosphonofluoridate (**1**), is a notoriously potent chemical warfare agent. The toxic effects of Soman result from irreversible inhibition of acetylcholinesterase (AChE) caused by phosphonylation of the active-site serine unit.[1] Strategies for protection against Soman involve pretreatment with a reversible cholinesterase inhibitor such as a carbamate as in the case of physostigmine, in which case the carbamylated enzyme protects a pool of AChE from phosphonylation by Soman.[2] Another approach involves use of pyridinium oxime nucleophiles to dephosphonylate the AChE ester.[3] A novel approach towards protection against Soman is creation of a monoclonal antibody against this molecule, which could either act as a scavenger or as a catalyst for its hydrolysis.[4] Soman (**1**) possesses two chiral centers, one at carbon and the other at phosphorus, and the four stereoisomers have been separated using gas chromatography on a chiral column.[5] There were designated 1-C(+)P(+), 1-(-)P(-), 1-C(-) P(+), and 1-C(+)P(-). The absolute configurations at carbon were deduced to be 1-C(+)P(+) = C(S)P(+), 1-(-)P(-) = C(R)P(-), 1-C(-) P(+) = C(R)P(+), and 1-C(+)P(-) = C(S)P(-) by synthesizing the pairs of diastereomers starting with (R)- and (S)-3,3-dimethyl-2-butanol.[6] The configuration at phosphorus was not assigned in this study. These stereoisomers exhibit different rate constants for inhibition of AChE (electric eel) and the enzymatic activity also correlates with their toxicity.[5]



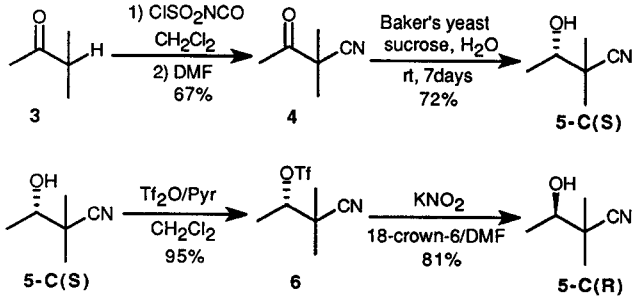
1 (Soman)



2

In order to produce an antibody for Soman, we chose the Soman simulant **2** as a hapten based upon a three-fold rationale. The hydrocarbon backbone simulates the 3,3-dimethyl-2-butyl group of Soman including R and S configurational possibilities for the stereogenic C2 carbon atom, the primary amino group at C4 serves as a site for attachment of a linker chain for conjugation to proteins as required for the immunochemistry, and the phosphorus atom is chiral in the same sense as the analogous tetracoordinate phosphorus atom of Soman, with the methoxy group in place of the fluorine atom. Substitution of OCH_3 for F avoids the extreme toxicity of Soman and yields a molecule suitable for the synthetic immunochemical steps involved in antibody generation. Furthermore, stereoselective synthesis of each of the four stereoisomers of **2** enables a test of the stereospecificity of monoclonal antibodies for Soman stereoisomers and probably allows an independent assignment of the absolute configuration at carbon and phosphorus for the Soman stereoisomers.

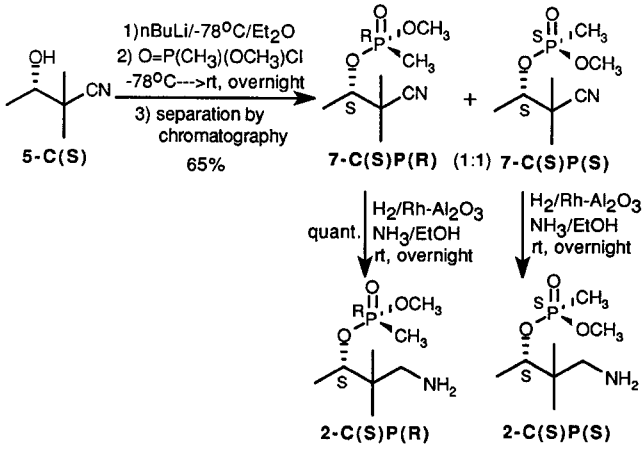
The starting point for the synthesis of the S and R isomers of **2** was to obtain the optically pure (S)- and (R)-2,2-dimethyl-3-hydroxybutanenitrile, **5-C(S)** and **5-C(R)**, respectively. Baker's yeast is known to reduce a variety of ketones enantioselectively.[7] Reduction of 2,2-dimethyl-3-oxobutanenitrile (**4**), obtained by treatment of **3** with chlorosulfonyl isocyanate,[8] with Baker's yeast gave the S alcohol **5-C(S)** exclusively in 72% yield.[9] The enantiomer **5-C(R)** was obtained by inversion of configuration of **5-C(S)** using the Moriarty procedure (Scheme 1).[10]



Scheme 1

Phosphonylation of **5-C(S)** was achieved by treatment of **5-C(S)** with n-BuLi and then methyl methylphosphonochloridate (Scheme 2). The reaction yielded a 1:1 mixture of diastereomers **7-C(S)P(R)** and **7-C(S)P(S)** which were separated by column chromatogaphy on silica gel with 2-propanol/hexane (1:9) as the eluent. The configuration at phosphorus for oily **7-C(S)P(S)** and **7-C(S)P(R)** was determined by X-ray analysis of their crystalline derivatives **9-C(S)P(S)** and **9-C(S)P(R)**, respectively (see below). Phosphonates **7-C(R)P(R)** and **7-C(R)P(S)** were obtained in the same manner starting from **5-C(R)**.

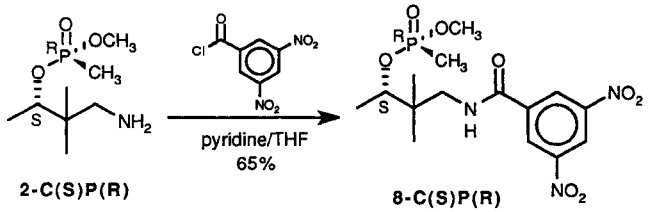
Hydrogenation of **7-C(S)P(R)** over rhodium (on alumina) in NH_3/EtOH afforded hapten **2-C(S)P(R)** quantitatively as an oil (Scheme 2).[11] The other amino phosphonate haptens **2-C(S)P(S)**, **2-C(R)P(R)**, and **2-C(R)P(S)** were prepared in the same manner from their corresponding cyano phosphonates **7-C(S)P(S)**, **7-C(R)P(R)**, and **7-C(R)P(S)**, respectively.



Scheme 2

In order to establish the absolute configuraion at phosphorus in the series of Soman simulants, a crystalline 3,5-dinitrobenzamide derivative for an X-ray analysis was made. Amino phosphonate **2-C(S)P(R)** was reacted with 3,5-dinitrobenzoyl chloride and pyridine in THF and the product was chromatographed on silica gel with acetone/petroleum ether (1:3) to give yellow crystalline **8-C(S)P(R)** (Scheme 3). Since the

absolute configuration of C2 of **8-C(S)P(R)** is S, inspection of the X-ray structure (not shown) reveals the R configuration at phosphorus. Thus, the stereochemical assignment of amino phosphonate **2-C(S)P(R)** and **7-C(S)P(R)** was established.

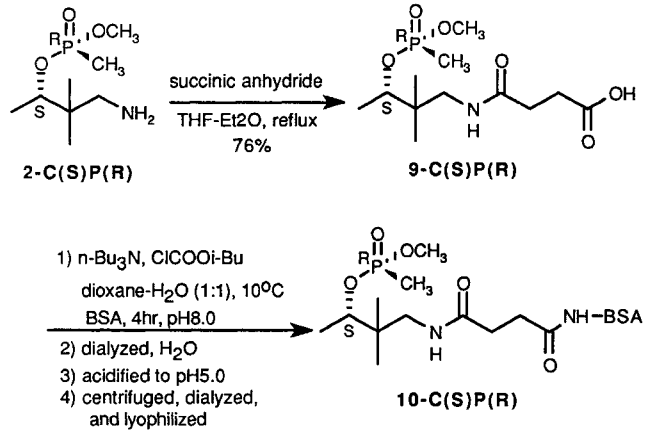


Scheme 3

The C(S)P(S) 3,5-dinitrobenzamide derivative **8-C(S)P(S)** was prepared in the same way and its configuration at phosphorus was determined by X-ray analysis (not shown). The configurations at phosphorus for **2-C(S)P(S)** and **7-C(S)P(S)** were then established.

The configurations of the C(R) series **2-C(R)P(R)**, **2-C(R)P(S)**, **7-C(R)P(R)**, and **7-C(R)P(S)** were established by comparing their ^{31}P NMR chemical shifts with those of the C(S) series **2-C(S)P(S)**, **2-C(S)P(R)**, **7-C(S)P(S)**, **and 7-C(S)P(R)**, respectively, since enantiomers have the same chemical shift in ^{31}P NMR spectroscopy.

To generate antibodies by immunization, the haptens **2** were conjugated to carrier proteins with succinyl group as a spacer. When amino phosphonate **2-C(S)P(R)** was refluxed with succinic anhydride in THF/Et_2O(1:1) overnight, succinamido derivative **9-C(S)P(R)** was obtained as an oil in 76% yield (Scheme 4). **9-C(S)P(S)**, **9-C(R)P(S)**, and **9-C(R)P(R)** derivatives were prepared analogously.



Scheme 4

9-C(S)P(R) was conjugated to HSA and BSA using the mixed anhydride method.[12] By reaction with isobutylchloroformate and tributylamine, succinamido derivative **9-C(S)P(R)** was converted in situ to an acid anhydride which then reacted with the amino groups of the proteins. After exhaustive dialysis and lyophilization, ^{31}P NMR spectroscopy was used to determine the amount of conjugated hapten with dimethyl methylphosphonate as a standard. The coupling reaction to each protein carrier was carried out at three different hapten-to-carrier ratios (see Table 1). The amount of conjugated hapten increased as the hapten-to-carrier ratio increased.

The spectroscopic data[13] of the C(S) isomers of compound **2**, **7**, and **9** are presented in "References and Notes." The hapten-protein conjugates **10** will be used for monoclonal antibody generation.

Table 1. Conjugation of Succinamido Phosphonates **9-C(S)P(R)** and **9-C(S)P(S)** to BSA and HSA

Amount of succinamido hapten used for conjugation (mg)		Amount of protein (mg)[a]	Amount of conjugated hapten[b]
9-C(S)P(R)	a) 53	400, BSA	23
	b) 80	400, BSA	26
	c) 133	400, BSA	45
9-C(S)P(R)	a) 200	1500, HSA	6
	b) 300	1500, HSA	9
	c) 500	1500, HSA	24
9-C(S)P(S)	a) 53	400, BSA	14
	b) 80	400, BSA	20
	c) 133	400, BSA	25
9-C(S)P(S)	a) 53	400, HSA	25
	b) 80	400, HSA	40
	c) 133	400, HSA	40

[a] BSA stands for bovine serum albumin and HSA for human serum albumin.

[b] Refer to moles of hapten present in the conjugate per mole of protein used, determined by ^{31}P NMR (81MHz, DMSO-d_6) with 85% H_3PO_4 as an external reference and dimethyl methylphosphonate as an internal standard.

References and Notes

(1) Koelle, G. B. *Handbuch der Agents*; Springer: Berlin, 1963; Vol. 15.

(2) (a) Koster, R. *J. Pharmac. Exp. Ther.* **1946**, *88*, 39. (b) Green, L. A. *Biochem. Pharmac.* **1983**, *32*, 1717. (c) Aldridge, W. N. in *Enzyme Inhibitors as Drugs*, Ed. M. Sandler, Macmillan, London, 1980. (d) Gearien, J. E. in *Medicinal Chemistry*, Ed. Burger, A., pp1307-1310, New York, Wiley, 1970. (e) Lennox, E. J.; Harris, L. W.; Talbot, B. G.; Anderson, D. R. *Life Sciences* **1985**, *37*, 793.

(3) (a) Koelle, G. B. *Handbuch der Experimentellen Pharmakologie*; Springer-Verlag, Berlin, 1963; Vol. 12. (b) Gray, A. P. *Drug Metabolism Reviews* **1984**, *15*, 557.

(4) (a) Jacobs, J.; Schultz, P. E.; Sugasawara, R.; Powell, M. *J. Am. Chem. Soc.* **1987**, *109*, 2574. (b) Pollack, S. J.; Jacobs, J. W.; Schultz, P. E. *Science* **1986**, *234*, 1570. (c) Napper, A. D.; Benkovic, S. J.; Tramontano, A.; Lerner, R. A. *Science* **1987**, *237*, 1041. (d) Janda, K. D.; Benkovic, S. J.; Lerner, R. A. *Science* **1989**, *244*, 437.

(5) Benschop, H.P.; Konings, C. A. G.; Van Genderen, J.; De Jong, P. A. *Toxicol. Appl. Pharmacol.* **1984**, *72*, 61.

(6) Degenhardt, C. E. A. M.; Van Den Berg, G. R.; De Jong, L. P.A.; Benschop, H. P.; Van Genderen, J.; Van De Meent, D. *J. Am. Chem. Soc.* **1986**, *108*, 8290.

(7) (a) Hoffman, R. W.; Ladner, W.; Steinbach, L.; Massa, W.; Schmidt, R.; Snatzke, E. *Chem. Ber.* **1981**, *114*, 2786. (b) Seuring, B.; Seebach, D. *Helv. Chim. Acta* **1975**, *60*, 1175. (c) Zuger, M. F.; Brenne, M. J. *Helv. Chim. Acta* **1984**, *67*, 1843.

(8) Rasmussen, J. K.; Hassner, A. *Synthesis* **1973**, 682.

(9) Balthazor, T. M.; Flores, R. A. *J. Org. Chem.* **1980**, *45*, 529.

(10) Moriarty, R. M.; Zhuang, H.; Penmasta, R.; Liu, K.; Awasthi, A. K.; Tuladhar, S. M.; Rao, M. S. C.; Singh, V. K. *Tetrahedron Lett.* **1993**, *34*, 8029.

(11) Freifelder, M. *J. Am. Chem. Soc.* **1960**, *82*, 2386.

(12) (a) Erlanger, B. F.; Borek, F.; Beiser, S. M.; Lieberman, S. *J. Biol. Chem.* **1958**, *228*, 713. (b) Erlanger, B. F. in *Methods in Enzymology*; Academic Press, New York, 1980; Vol. 70, p. 85.

(13) **7-C(S)P(R)**: ^{1}H NMR ($CDCl_3$): 1.34 (s, 3H), 1.39 (s, 3H), 1.47 (d, J=6.26Hz, 3H), 1.53 (d, J=17.63Hz, 3H), 3.72 (d, J=11.2Hz, 3H), 4.40 (m, 1H); ^{13}C NMR ($CDCl_3$): 10.87 (d, J=147.1Hz), 17.88, 22.88, 23.47, 38.27, 52.12 (d, J=6.0Hz), 76.23 (d, J=6.7Hz), 122.66; ^{31}P NMR ($CDCl_3$): 32.57ppm. **7-C(S)P(S)**: ^{1}H NMR ($CDCl_3$): 1.34 (s, 3H), 1.40 (s, 3H), 1.45 (d, J=6.26Hz, 3H), 1.52 (d, J=17.62Hz, 3H), 3.76 (d, J=11.1Hz, 3H), 4.39 (m, 1H); ^{13}C NMR ($CDCl_3$): 11.76 (d, J=145.9Hz), 17.58, 22.82, 23.25, 38.14, 52.35 (d, J=6.6Hz), 76.43 (d, J=6.6Hz), 122.58; ^{31}P NMR ($CDCl_3$): 31.64ppm. **2-C(S)P(R)**: ^{1}H NMR ($CDCl_3$): 0.87 (s, 3H), 0.92 (s, 3H), 1.27 (d, J=6.41Hz, 3H), 1.47 (d, J=17.55Hz, 3H), 2.49 (d, J=13.16Hz, 1H), 2.72 (d, J=13.16Hz, 1H), 3.4 (br s, 2H), 3.71 (d, J=11.15Hz, 3H), 4.49-4.52 (m, 1H); ^{31}P NMR ($CDCl_3$): 32.26ppm. **2-C(S)P(S)**: ^{1}H NMR ($CDCl_3$): 0.88 (s, 3H), 0.89 (s, 3H), 1.29 (d, J=6.50Hz, 3H), 1.48 (d, J=17.64Hz, 3H), 1.5 (br s, 2H), 2.45 (d, J=13.40Hz, 1H), 2.74 (d, J=13.40Hz, 1H), 3.75 (d, J=11.17Hz, 3H), 4.44-4.50 (m, 1H); ^{31}P NMR ($CDCl_3$): 31.53ppm. **9-C(S)P(R)**: ^{1}H NMR ($CDCl_3$): 0.87 (s, 3H), 0.90 (s, 3H), 1.27 (d, J=6.42Hz, 3H), 1.53 (d, J=13.75Hz, 3H), 2.57-2.61 and 2.65-2.69 (m, 4H), 2.82 (dd, J=13.75 & 4.12Hz, 1H), 3.55 (dd, J=13.77 & 9.00Hz, 1H), 3.76 (d, J=11.12Hz, 3H), 4.45-4.49 (m, 1H), 7.43 (br s, 1H); ^{13}C NMR ($CDCl_3$): 10.36 (d, J=146.77Hz), 16.28, 19.23, 21.82, 30.47, 31.06, 39.24, 46.60, 53.00 (d, J=5.49Hz), 76.15 (d, J=7.64Hz), 173.15, 174.95; ^{31}P NMR ($CDCl_3$): 34.98ppm. **9-C(S)P(S)**: ^{1}H NMR ($CDCl_3$): 0.88 (s, 3H), 0.94 (s, 3H), 1.26 (d, J=6.39Hz, 3H), 1.55 (d, J=17.35Hz, 3H), 2.57-2.63 and 2.66-2.71 (m, 4H), 2.89 (dd, J=14.02 & 4.10Hz, 1H), 3.56 (dd, J=13.74 & 9.00Hz, 1H), 3.75 (d, J=11.25Hz, 3H), 4.40-4.44 (m, 1H), 7.33 (br s, 1H); ^{13}C NMR ($CDCl_3$): 11.44 (d, J=143.17Hz), 16.28, 19.37, 21.86, 30.28, 31.10, 39.40, 46.36, 52.25 (d, J=6.52Hz), 77.18 (d, J=6.33Hz), 172.88, 175.14; ^{31}P NMR ($CDCl_3$): 33.83ppm.