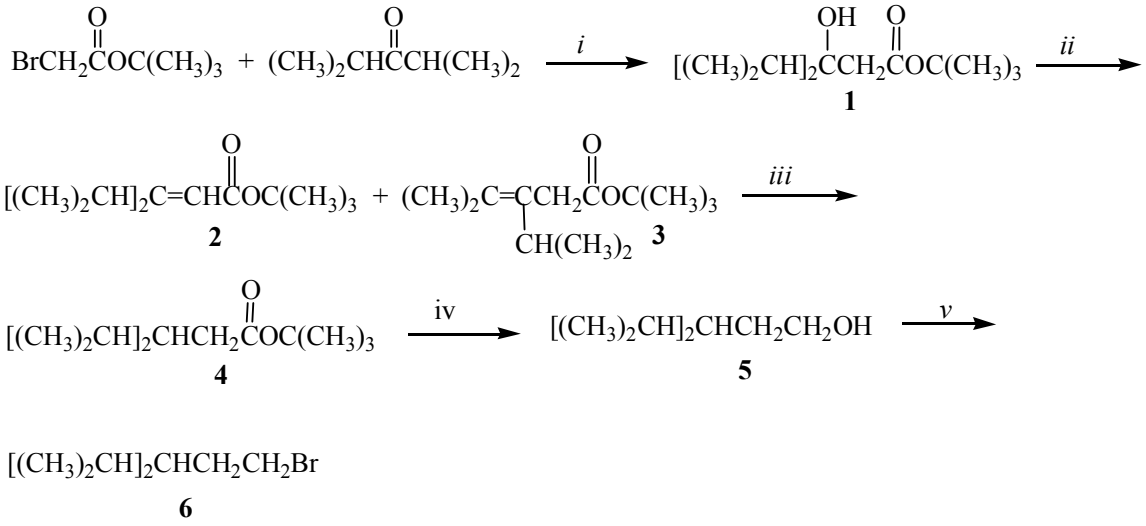
Stereoselectivity Toward VX is Determined by Interactions with Residues of the Acyl Pocket as well as of the Peripheral Anionic Site of AChE

Suplementary Material

Synthesis of nc-VX Enantiomers

The VX "non-charged" analogs were synthesized as outlined in schemes 1,2

Scheme 1



i. 3-Hydroxy-3-isopropyl-4-methyl-pentanoic acid *tert*- butyl ester (**1**)

Zinc powder (11.35 g, 0.175 mol) and iodine (50 mg) were refluxed in dry THF for 1 hr. A mixture of *tert*- butyl bromoacetate (22.5 g, 0.115 mol) and 2,4-dimethyl-3-one (11.4 g, 0.1 mol) in dry THF (80 mL) was added dropwise over a period of 30 min and the solution was further refluxed for 3 hr. The solution was cooled down to rt and the pH was adjusted to 1 by addition of hydrochloric acid. The precipitate was filtered off, the filtrate was washed 3 times with ethyl acetae. The organic layer was extracted with 5% aqueous sodium carbonate (400 mL), water (400 mL), dried (Na_2SO_4) and finally concentrated *in vacuo* to give a colorless liquid (68%).

^{1}H NMR ($CDCl_3$): 0/95 (dd, J=7 Hz, 12H), 1.50 (s, 9H), 1.95 (m, 2H), 2.36 (s, 2H), 4.50 (s, 1H).

^{13}C NMR: 17.3, 17.5, 27.9, 34.9, 36.3, 81.5, 174.3 .

ii. 3-Isopropyl-4-methyl-pent-2-enoic acid *tert-* butyl ester (**2a**) and 3-Isopropyl-4-methyl-pent-3-enoic acid *tert-* butyl ester (**3**)

Phosphorus oxychloride (5.3 g, 0.034 mol), **1** (4.73 g, 0.026 mol) and pyridine (27 mL) were stirred for 6 hr at rt. The reaction propagation was followed up by TLC (silica, ether:hexane 3:1). Cold water was added carefully and the solution was extracted with ether (500 mL). The organic layer was washed successively with 1N HCl (300 mL) and 5% $NaHCO_3$ (200 mL), dried (Na_2SO_4) and the solvent removed *in vacuo*. The crude product was obtained (in 72% yield) as a 1:1 isomeric mixture of **2,** and used for the next reaction without further purification.

^{1}H NMR (**2** , $CDCl_3$): 1.02 (dd, J=6.7 Hz, 12H), 1.40 (s, 9H), 2.48 (m, 2H), 5.52 (s, 1H).

^{1}H NMR (**3**): 0.89 (d, 6H), 1.45 (s, 9H), 1.63 (s, 3H), 1.69 (s, 3H), 2.80 (m, 1H), 2.84 (s, 2H).

iii. 3-Isopropyl-4-methyl-pentanoic acid *tert-* butyl ester (**4**)

tert- Butyl esters **2** (900 mg, 4.24 mmol) were reduced in methanol (80 mL) in the presence of Palladium (310 mg) at 50 psi H_2 for 4 hr at rt. The mixture was filtered and the solvent removed *in vacup* to give 3 as a colorless liquid (80%).

^{1}H NMR ($CDCl_3$): 0.86 (dd, 12H), 1.44 (s, 9H), 1.57 (m, 1H), 1.73 (m, 2H), 2.07 (d, 2H).

^{13}C NMR: 17.8, 18.7, 28.1, 29.4, 34.3, 46.8, 79.8, 174.3 .

iv. 3-Isopropyl-4-methyl-pentan-1-ol (**5**)

Ester **4** (720 mg, 0.018 mol) in dry ether (5 mL) was added dropwise to a slurry of $LiAlH_4$ (700 mg, 0.018 mol) in ether (150 mL). The mixture was stirred for 15 hr at rt, 1N HCl (50 mL) was added carefully and the solution was extracted with ether (100 mL). The organic layer was dried (Na_2SO_4) and the solvent removed *in vacuo* to give **5** as a colorless liquid (90%).

^{1}H NMR ($CDCl_3$): 0.86 (dd, 12H), 1.48 (m, 3H), 1.73 (m, 2H), 3.60 (t, 2H)

^{13}C NMR: 19.1, 21.4, 29.4, 31.1, 46.4, 63.8 .

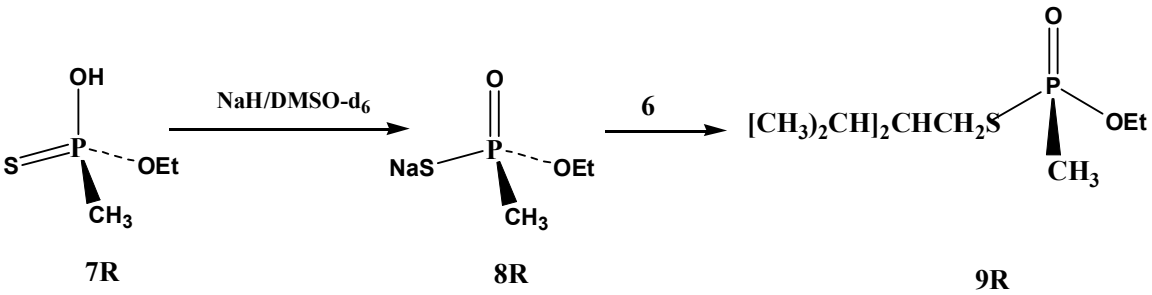
v. 3-(2-Bromoethyl)-2,4-dimethylpentane (**6**)

Alcohol **5** (390 mg, 2.7 mmol) and phosphorus tribromide (783 mg, 2.9 mmol) were heated in C_6H_6 (20 mL) for 5 days at 85 ^{0}C. the solvent was removed *in vacuo* and $CHCl_3$ (50 mL) was added. The solution was washed with aqueous potassium carbonate, dried (Na_2SO_4) and the solvent removed *in vacuo*. The resulting colorless oil was purified by column chromatography (silica, ether: hexane 3:1) to give pure **6** (18%).

^{1}H NMR ($CDCl^3$): 0.78 (dd, 12H), 1.56 (m, 3H), 1.70 (m, 2H), 3.16 (t, 2H).

^{13}C NMR: 18.9, 21.1, 29.0, 31.8, 33.7, 49.3 .

Scheme2



7R **8R** **9R**

O-Ethyl methylphosphonothioate sodium salt (**8R** and **8S)**

Optically active **7** (30 mg, 0.4 mmol, each enantiomer prepared following the above procedure) and NaH (6 mg, 0.25 mmol) were stirred for 30 min at rt in DMSO-d6 (300 μL). The reaction propagation was followed up by ^{31}P NMR. The resulting salts **7** were further used as such in the final reaction.

^{1}H NMR (6R or 6S, DMSO-d6): 1.23 (t, 3H), 1.45 (d, 14.6 Hz, 3H), 3.82 (m, 2H).

^{31}P {^{1}H} NMR (6R or 6S, DMSO-d6): 72.7

O-Ethyl S-(3-isopropyl-4-methylpentyl) methylphosphonothioate (**9R** and **9S**)

The preparation of optically active **9R** or **9S** was carried out in an NMR tubeon mixing either **8R** or **8S** (from step e) with bromide **5** (38 mg, 0.19 mol) and heating up the tube to 40 ^{0}C for 48 hr. Each solution was poured into a separatory funnel containing 5% aqueous sodium bicarbonate (10 mL) and extracted with ether (10 mL). After drying (Na_2SO_4) the solvents were removed at 8 mmHg and the crude product was purified by column chromatography (ether:hexane 1:1, 40% yield).

Products **9R** and **9S** were obtained >95% enantiomerically pure as evidenced from ^{1}H NMR using the chiral reagent [R]-222-trifluoro 1-(9-anthryl) ethanol.

^{1}H NMR ($CDCl_3$, **9R** or **9S**): 0.86 (dd, 12H), 0.92 (t, 1H), 1.33 (t, 3H), 1.55 (m, 2H), 1.70 (m, 2H), 1.77 (d, J=15.6 Hz, 3H), 2.81 (m, 2H), 4.11 (m, 2H).

^{13}C NMR (**9R** or **9S**): 16.1 (d, J_{PC}=7.2 Hz), 19.1 20.1 (d, J_{PC}=110Hz), 21.3 (d, J_{PC}=3.2 Hz), 29.0, 29.7 (d, J_{PC}=4.9 Hz), 31.1 (d, J_{PC}=2.9 Hz), 49.9, 61.1 (d, J_{PC}=6.8 Hz).

^{31}P NMR (**9R** or **9S**): 50.6 ppm.