Bioorganic & Medicinal Chemistry Letters, Vol.2, No 12, pp 1547-1550, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

SYNTHESIS OF CHIRAL PHOSPHORUS MUSTARDS DERIVED FROM SERINE

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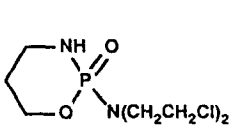
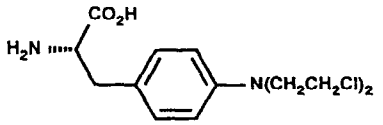
(*Received* 21 *July* 1992)

Abstract: The synthesis and biological evaluation of chiral, diastereomeric phosphorus mustards derived from natural and unnatural serine are reported herein.

Cyclophosphosphamide (1) and melphalan (2) are successful members of the class of anti-proliferative agents known as mustards. Both compounds initiate their action as

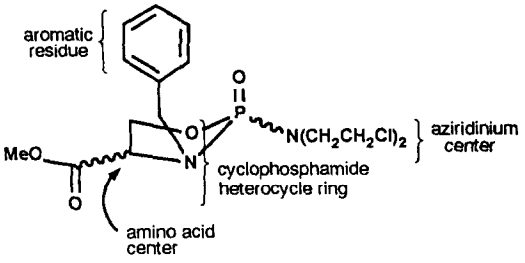


1: cyclophosphamide



2: melphalan

chemotherapeutics by alkylation of an essential purine or pyrimidine residue of DNA by the preformed aziridinium (mustard) ring, although alternate reactions may occur. Following alkylation of DNA, fundamental mechanisms of DNA replication are impaired leading to a reduced capacity of the rapidly proliferating cancer cell to undergo normal mitosis and hence, cell division.[1] Despite extensive development of lead compounds bearing unique functional group arrays with promising ***in vitro*** activity, the nitrogen mustards, in particular **1**, remain dominant chemotherapeutic and immunosuppressive[2] agents. However, nitrogen mustards also have serious drawbacks including: 1. the alkylation of normal cells, 2. resistance, 3. instability and, 4. problems in cellular uptake. Owing to these liabilities, the number of clinically active agents remains quite small,[3] providing the impetus for further exploration of new and effective antitumor compounds. Based upon the continued clinical success and unique metabolism of **1**,[4] the active transport attributes of **2**,[5] and our own research with chiral phosphorus molecules we set out to

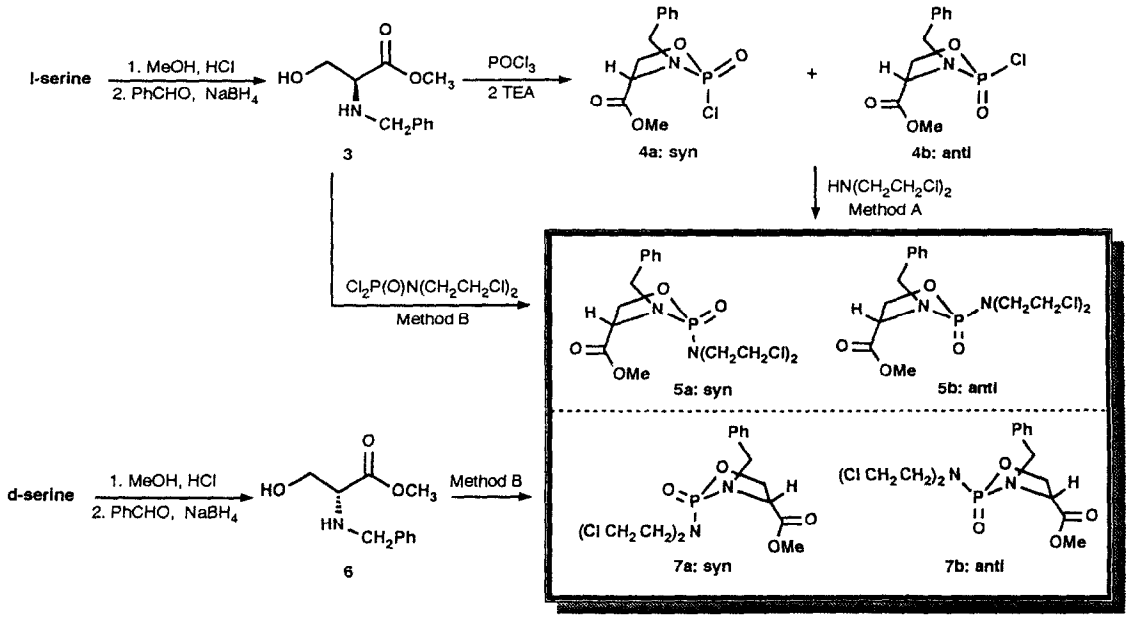


prepare a new class of anti-proliferative agents (below). In particular, we hoped to unite the influences of the heterocyclic skeleton of **1**, the amino acid recognition element of **2**, the mustard moiety, and an aromatic moiety (to aid intercalation) into one molecule.

Guided by these features, we also intended to probe any possible involvement of stereochemistry upon the interaction of these analogues with cancer cells since studies with the enantiomers of **1** and **2** remain perplexing. For example, melphalan (**2**), medphalan (from the d-isomer) and racemic **2** have approximately equal potencies.[6] Cyclophosphamide (**1**) is generally administered as the racemate. However, an early study by Cox et al.[7] showed that optically enriched **1** was excreted following administration of racemic **1**. Suggestive that a single enantiomer of **1** might have enhanced anti-cancer activity or reduced toxicity, this study prompted further synthetic investigation.[8] Metabolic studies of **1** conducted *in vitro* and *in vivo* revealed that there was a species-dependent, stereoselective oxidation to 4-hydroxy-**1**, although animal tumor test systems indicated no therapeutic advantage for single enantiomer administration.[9] Despite a lack of proof for single enantiomer influences, the interplay between an amino acid and phosphorus stereocenter (a diastereomer relationship) has not been thoroughly examined.[10]

Herein, we wish to report on the synthesis, anti-cancer and anti-HIV activity of four, chiral, phosphorus mustard 1,3,2-oxazaphospholidin-2-ones (OAP's) **5a**, **5b**, **7a**, and **7b** from *l*- and *d*-serine, respectively (Scheme I). Previously, we had prepared phosphorus ester OAP's from *l*-

SCHEME I



serine,[11] and this general methodology was used for the preparation of the target molecules **5ab** and **7ab**. *l*-Serine was first converted to the N-benzyl methyl serinoate[11] and reacted with phosphorus oxychloride ($POCl_3$) to furnish a diastereomeric pair of 2-chloro oxazaphospholidin-2-ones **4a** and **4b**.[11] Without further purification, **5a** (S_CS_P) and **5b** (S_CR_P) were formed by reaction with excess bis-(2-chloroethyl)amine, albeit in a modest 43% yield (Method A).[12]

A more efficient (68-77%) route reacted the N-benzyl methyl serinoate derivative **3** or **6** with bis-(2-chloroethyl)amino phosphoric dichloride[13] (Method B; Scheme I) to provide directly the diastereomeric phosphorus mustards **5ab** or **7ab**,[14] following chromatographic separation.

Two facets of the stereochemistry required our attention: the relationship between the exocyclic bis-chloroethylamine ligand and the carbomethoxy group, and the assignment of the enantiomer pairs. Previously, we found that the P-31 NMR absorbance appears at a greater chemical shift (lower field) when the syn orientation of the carbomethoxy and exocyclic ligand is indicated.[11] By analogy, **5a** and **7a** (δ P-31 = 27.8 ppm) have the syn orientation and **5b** and **7b** (δ P-31 = 25.4 ppm) have the anti orientation. Optical rotations both confirmed this relationship and established the enantiomers as **5a** ([α] = -2.34) and **7a** ([α] = +3.94), and **5b** ([α] = -42.48) and **7b** ([α] = +47.24). Although our prior study also indicated a correlation of the carbonyl chemical shift with diastereomer orientation (lower field = anti orientation), this trend was reversed for the title compounds.

Results: Screening by the National Cancer Institute showed that all four diastereomers have very little *in vitro* activity against a wide variety of tumor cell lines. Although none of the diastereomers were active enough to warrant further testing, the stereochemistry at both phosphorus and carbon seemed to have a slight influence on the biological activity. Anti-HIV testing showed no activity.

Acknowledgments: The authors wish to acknowledge kind financial support from the National Institutes of Health (ESO4434), Loyola University of Chicago for the purchase of the Varian VXR-300 MHz NMR used in this study, and the NCI for the biological evaluation of compounds **5a/b** and **7a/b**.

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12. **Methyl (2*S*, 4*S*) and (2*R*, 4*S*)-2-[bis-(2-chloroethyl)amino]-2-oxo-3-benzyl-1,3,2-oxazaphospholidin-4-carboxylate (5a and 5b). Method A.** To a solution of methyl (2*S*, 4*S*)- and (2*R*, 4*S*)-2-chloro-2-oxo-3-benzyl-1,3,2-oxazaphospholidin-4-carboxylate[11] (132 mg, 0.46 mmol) in 5 mL of dry toluene was added bis-(2-chloroethyl)amine hydrochloride (407 mg, 2.28 mmol) followed by the addition of triethylamine (0.32 mL, 2.28 mmol) at RT. After stirring overnight, the reaction mixture was filtered through a frit containing a 1 cm layer of Celite. The solvent was removed by rotary evaporation, and the crude oil was purified by flash chromatography (silica gel, 100% ether), producing the fast (**5a**; R_f=0.13) and slow (**5b**; R,0.06) isomers in 65.2 and 10.3 mg (43.1%), respectively.

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14. **Method B.** To a solution of bis-(2-chloroethyl)aminophosphoric dichloride (450 mg, 1.74 mmol) in 10 mL of dry toluene was added a solution of (*S*)-N-benzyl methyl serinoate (364 mg, 1.74 mmol) in 10 mL of dry toluene at RT. To the stirred mixture was added triethylamine (0.49 mL, 3.48 mmol). After stirring overnight, the reaction mixture was filtered through a frit containing a 1 cm layer of Celite, and the solvent was removed en vacuo. Purification by flash chromatography produced **5a** (278 mg) and **5b** (260 mg) in 78% overall yield. Anal. Calcd for $C_{15}H_{21}N_2O_4PCl_2$: C, 45.59; H, 5.36; N, 7.09. Found: C, 45.72; H, 5.63; N, 6.99. **5a (syn)**: $[\alpha]_D^{21}$ = -2.34 (c = 0.725, $CHCl_3$). ^{1}H NMR: δ 7.47-7.44 (m, 2), 7.38-7.28 (m, 3), 4.38 (ddd, 1, *J* = 9.55, 8.32, 3.15 Hz), 4.43 (dd, 1, *J* = 14.40, 9.43 Hz), 4.27 (ddd, 1, *J* = 19.02, 9.57, 3.02 Hz), 4.10 (dd, 1, *J* = 14.60, 11.72 Hz), 3.87-3.81 (m, 1), 3.75 (s, 3), 3.67-3.37 (m, 8). ^{13}C NMR: δ 171.44 (d, *J* = 5.6 Hz), 135.57 (d, *J* = 1.6 Hz), 128.88 (2), 128.69 (2), 128.04, 65.06, 56.49 (d, *J* = 18.0 Hz), 52.63, 49.27, (d, *J* = 5.2 Hz), 47.43 (d, *J* = 5.7 Hz), 42.31. ^{31}P NMR: δ 27.83. **5b (anti)**: $[\alpha]_D^{21}$ = -42.48 (c = 0.605, $CHCl_3$). ^{1}H NMR: δ 7.40-7.28 (m, 5), 4.41-4.23 (m, 4), 3.93 (ddd, 1, *J* =16.64, 7.60, 2.68 Hz), 3.72 (s, 3), 3.70-3.47 (m, 6), 3.41-3.29 (m, 2). ^{13}C NMR: δ 170.63, 135.69 (d, *J* = 6.0 Hz), 128.82 (2), 128.76 (2), 128.05, 66.58, 57.64 (d, *J* = 16.1 Hz), 52.58, 49.36 (d, *J* = 4.6 Hz), 46.86 (d, *J* = 6.1 Hz), 42.35. ^{31}P NMR: δ 25.42.

Methyl (2*R*, 4*R*) and (2*S*, 4*R*)-2-[bis-(2-chloroethyl)amino]-2-oxo-3-benzyl 1,3,2-oxazaphospholidine-4-carboxylate (7a and 7b). Method B was repeated using (*R*)-N-benzyl methyl serinoate to produce **7a** and **7b** in 77% isolated yield. **7a (syn)**: $[\alpha]_D^{21}$ = +3.94 (c = 0.710, $CHCl_3$). **7b (anti)**: $[\alpha]_D^{21}$ = +47.24 (c = 0.635, $CHCl_3$). ^{1}H, ^{13}C, and ^{31}P NMR were identical to respective fast and slow bands **5a** and **5b**. Anal. Calcd for $C_{15}H_{21}N_2O_4PCl_2$: C, 45.59; H, 5.36; N, 7.09. Found: C, 45.74; H, 5.52; N, 7.09.