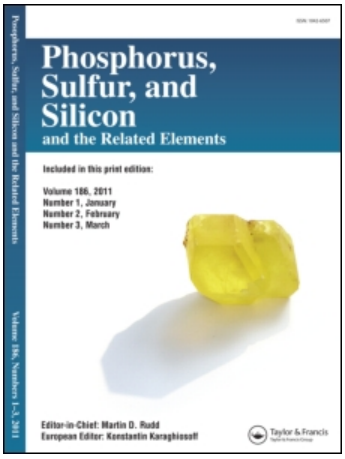

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Communication

STEREOSELECTIVE INHIBITION OF CHOLESTEROL ESTERASE BY ENANTIOMERIC PHOSPHONATES

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Enantiomers of methyl 2-naphthyl hexylphosphonate (MNHP) were prepared via diastereomeric phosphonamidate intermediates. The enantiomers exhibit stereoselective inhibition of purified porcine pancreatic cholesterol esterase; the first-order inhibition rate constant of the (−)-isomer is approximately fourfold higher than the rate constant of the (+)-isomer.

Keywords: Cholesterol esterase; phosphonate; enzyme inhibitor; chiral; stereoselective

INTRODUCTION

Pancreatic cholesterol esterase (CEase) [EC 3.1.1.13] catalyzes the hydrolysis of cholesteryl esters, triglycerides, and phospholipids in the intestinal lumen.[1] It has been shown that removal of the enzyme from pancreatic juice results in an 80% reduction of absorption of cholesterol into the bloodstream of rats.[2] Since serum cholesterol levels are correlated with atherosclerosis[3], development of CEase inhibitors may be of potential therapeutic value.

CEase catalyzes the *in vitro* hydrolysis of *p*-nitrophenyl butyrate via an acylenzyme mechanism[4,5] that is similar to the mechanism of serine proteases.[6] *p*-Nitrophenyl-N-butyl and N-octyl carbamates are potent active site-directed irreversible inhibitors of porcine pancreatic CEase, with second-order inhibition rate constants $>10^4$ $M^{-1}s^{-1}$.[7] Phosphates and phosphonates are also potent

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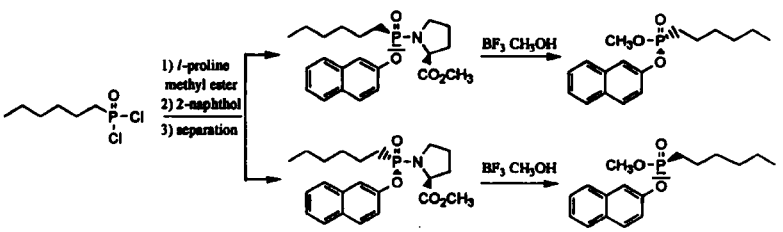
active site-directed irreversible inhibitors of the porcine enzyme. Diethyl *p*-nitrophenyl phosphate exhibits a second-order inhibition rate constant of 10^5 $M^{-1}s^{-1}$, while the second-order rate constant for racemic methyl 2-naphthyl hexylphosphonate (MNHP) is $>10^4 M^{-1}s^{-1}$.[8,9]

Acetylcholinesterase, an enzyme that is known to catalyze an acylenzyme mechanism, exhibits a variable chiral selectivity for enantiomeric methylphosphonothioates that depends on both the nature of the ester and of the thioic leaving groups and on the absolute configuration about the asymmetric phosphorus.[10] The phosphonate CEase inhibitors may also contain a chiral phosphorus. Therefore it is of interest to determine if this lipase exhibits a chiral selectivity for enantiomeric phosphonate inhibitors. This communication describes the previously unreported synthesis and assay of enantiomeric phosphonate CEase inhibitors.

EXPERIMENTAL AND RESULTS

The synthetic route to the enantiomeric phosphonates is shown in Scheme 1. Diastereomeric phosphoramidates were prepared using *l*-proline methyl ester as a chiral reagent.[11] *l*-Proline methyl ester (1.3 equiv.) in dry CH_2Cl_2 was added dropwise to hexylphosphonic dichloride (1 equiv.) in dry CH_2Cl_2 with triethylamine (2 equiv.) under argon and the reaction was carried out at room temperature for 4–5 hours. 2-Naphthol (1 equiv.) in dry CH_2Cl_2 was then added dropwise and stirred overnight. The crude reaction mixture was washed with water and extracted with ether. The diastereomers were separated with a Chromatotron® (preparative, centrifugally accelerated, radial thin-layer chromatograph) using silica gel and hexane:ethyl acetate (5:1) as eluant.



SCHEME 1

One diastereomer exhibits $[\alpha]_D^{20} = -35$ (ethyl ether); ^{31}P-NMR δ33.85(s); and ^{1}H-NMR ($CDCl_3$) δ0.97(t,3H), 1.25–2.11(m,14H), 3.25(m,2H), 3.67(s,3H), 4.43(m,1H), 7.29–7.82(m,7H). The other diastereomer is characterized by $[\alpha]_D^{20}$

= −72 (ethyl ether); ^{31}P-NMR ($CDCl_3$) δ33.32(s); and ^{1}H-NMR ($CDCl_3$) δ0.86(t,3H), 1.18–1.48(m,6H), 1.55–2.04(m,8H), 3.38(m,2H), 3.54(s,3H), 4.27(m,1H), 7.41(m,3H), 7.76(t,4H). Shifts in ^{31}P-NMR and ^{1}H-NMR are relative to phosphoric acid and TMS, respectively.

Boron trifluoride-methanol complex (10 equiv.) was added dropwise to the individual diastereomers in dry THF under argon and the reactions were carried out at room temperature overnight.[12] The reaction mixtures were washed with water, extracted with ether, and the enantiomers were separated from the crude reaction mixtures on silica gel with a Chromatotron®, using hexane/ether as eluant, to yield colorless oils. Specific rotation of one enantiomer (produced from the diastereomer with $[\alpha]_D^{20} = -35$) is $[\alpha]_D^{20} = +17$; the other enantiomer (produced from the diastereomer with $[\alpha]_D^{20} = -72$) is $[\alpha]_D^{20} = -18$. ^{31}P-NMR ($CDCl_3$) yielded a single peak for each enantiomer at δ31.79 relative to phosphoric acid. ^{1}H-NMR ($CDCl_3$) spectra of the enantiomers are identical, exhibiting the following δ0.88(t,J = 6.6,3H), 1.22–1.50(m,6H), 1.72(m,2H), 1.94(m,2H), 3.82(d,J = 10.9,3H), 7.32–7.85(m,7H). High resolution positive ion FAB-mass spectrometry yielded *m/z* 307.1463 $[M + H]^+$ for each enantiomer. The methanolysis reaction proceeded by a single mechanism with stereochemistry supported by a single peak in ^{31}P-NMR.

The individual enantiomers were assayed *in vitro* for inhibition of porcine pancreatic CEase purified by a published procedure.[13] The spectrophotometric assays used *p*-nitrophenyl butyrate (PNPB) as substrate.[7] Reactions were run in 0.10 M sodium phosphate buffer, pH 7.0, that contained 0.1 N NaCl. Acetonitrile solutions of PNPB and the MNHP inhibitors were added to buffer equilibrated at 25°C to give final concentrations of 100 mM and 10 mM, respectively. The concentration of acetonitrile was held constant at 2% v/v. The final concentration of CEase was 2.5 mg/mL. Triplicate sets of data were collected. First-order inhibition rate constants (k_{app}) were determined as previously described by fitting data to the following first-order function.[7] The k_{app} (s^{-1}) of (−)-MNHP is 1.76 E-2 ± 7.6 E-4; the k_{app} (s^{-1}) of (+)-MNHP is 4.77 E-3 ± 5.7 E-4.

$$A = (A_0 - A_\infty)^{-kt} + A_\infty$$

CONCLUSIONS

In conclusion, a method for the preparation of enantiomeric phosphonate inhibitors of CEase has been developed. Enantiomers can be synthesized via diastereomeric phosphonamidate intermediates. Furthermore, MNHP enantiomers ex-

hibit stereoselective inhibition of CEase, differing nearly four-fold in first-order inhibition rate constants. This finding is in general agreement with inhibition of another serine-esterase, acetylcholinesterase, by methylphosphonothioates.[10]

Cholesterol esterase is a unique target for inhibitors with therapeutic potential for lowering serum cholesterol. Developing carbamate and phosphonate inhibitors requires knowledge of the enzymology of CEase. These results provide knowledge necessary for continued development in this area. As with other classes of chiral therapeutic agents, knowledge of differential activities related to stereoisomerism is important with regard to dose, side effects, and metabolism.

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