2-Octyl-4H-1,3,2-benzodioxaphosphorin 2-Oxide Labelled with Tritium in the Octyl and Aryl Moieties

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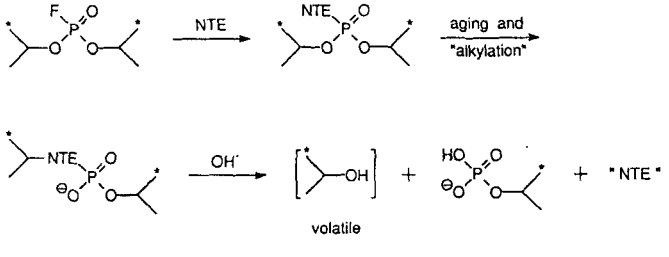
SUMMARY

2-Octyl-4H-1,3,2-benzodioxaphosphorin 2-oxide (octyl-BDPO) was labelled with tritium in the 7,8-octyl and 6-BDPO positions at specific activities of 38 and 16 Ci/mmol, respectively, by catalytic reductions of the corresponding olefin and bromo precursors with tritium gas. This potent mechanism-based inhibitor for neuropathy target esterase (NTE) (I_{50} = 0.25 nM) and delayed neurotoxicant (active in hens at 3 mg/kg) was designed to determine phosphorylated NTE which retains the octyl label and differentiate aging with or without intramolecular group transfer ("alkylation") reactions at the NTE active site from the fate of the aryl label.

Key Words: benzodioxaphosphorin, neuropathy target esterase, organophosphate-induced delayed neuropathy, tritium labelling

INTRODUCTION

Several types of organophosphorus compounds induce delayed neuropathy in humans by a mechanism proposed to involve phosphorylation and aging of a protein(s) designated neuropathy target esterase (NTE).[1] Current knowledge of NTE comes primarily from studies with the brain of hens, another sensitive species, in which the target reacts with [^{3}H]diisopropyl phosphorofluoridate as follows:[2-10]



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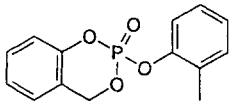
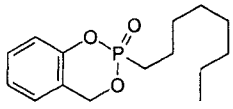
A similar scheme is proposed with [^{3}H]di-n-pentyl 2,2-dichlorovinyl phosphate.[11] These radioligands are of moderate to high potency as NTE inhibitors, with 50% inhibition values (I_{50}s) of 700 and 3 nM, respectively,[4] and they are also achiral dialkylphosphates and thereby represent only one type of active agent. In the process of aging of [^{3}H]diisopropylphosphoryl-NTE, one of the two [^{3}H]isopropyl groups undergoes intramolecular group transfer ("alkylation") to a site from which it can be liberated in volatile form ([^{3}H]isopropanol?) by treatment with alkali, providing the only specific radiochemical assay currently available for active site-labelled NTE.[5,10]

An improved radioligand for the NTE active site would combine several features: optimized potency for enhanced specificity; very rapid phosphorylation and aging reactions; two positions of labelling to differentiate between the phosphorylation and aging processes with or without "alkylation." This has been achieved in the 2-substituted-4H-1,3,2-benzodioxaphosphorin 2-oxides (BDPOs). This class of compounds became important when o-methylphenoxy-BDPO was recognized as the metabolic activation product of tri-o-cresyl phosphate, the causal agent for ~30,000 cases of human delayed neuropathy.[9,12]

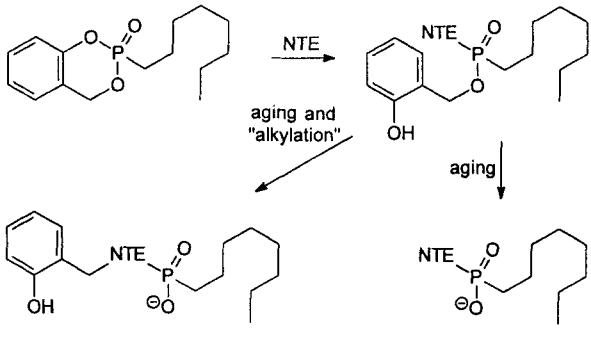


o-methylphenoxy - BDPO



octyl - BDPO

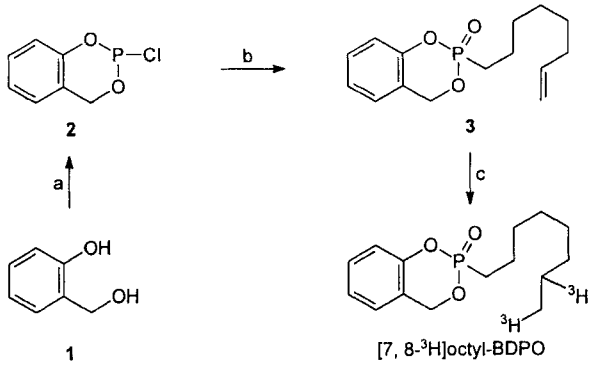
Structural optimization for potency in inhibition of NTE resulted in selection of octyl-BDPO as a preferred compound (I_{50} = 0.25 nM) with 240-fold greater activity in vitro than o-methylphenoxy-BDPO and it is also neuropathic in hens at 3 mg/kg.[13] The enantiomers of octyl-BDPO, separated on a chiral HPLC column, differ by 92-fold in inhibitory potency for NTE with *S* more potent than *R*.[14] NTE inhibition by octyl-BDPO involves rapid phosphorylation[15] and aging (by analogy with phenoxy-BDPO[6]). It is expected that the octylphosphonyl moiety will be



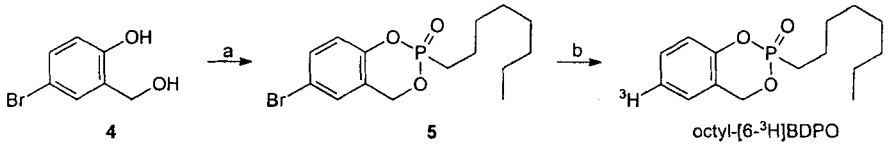
retained on the enzyme's active site and the fate of the 2-hydroxybenzyl group will be determined by whether subsequent reactions involve only aging or both aging and "alkylation". Tritium labelling in each of these two groups at high specific activity reported here provides suitable radioligands to further probe the mechanism of delayed neuropathy.

RESULTS AND DISCUSSION

The octyl and aryl groups of octyl-BDPO were chosen as sites for tritium labelling in two separate preparations to facilitate studies on phosphorylation, aging and possible "alkylation". The reaction sequences are given below.

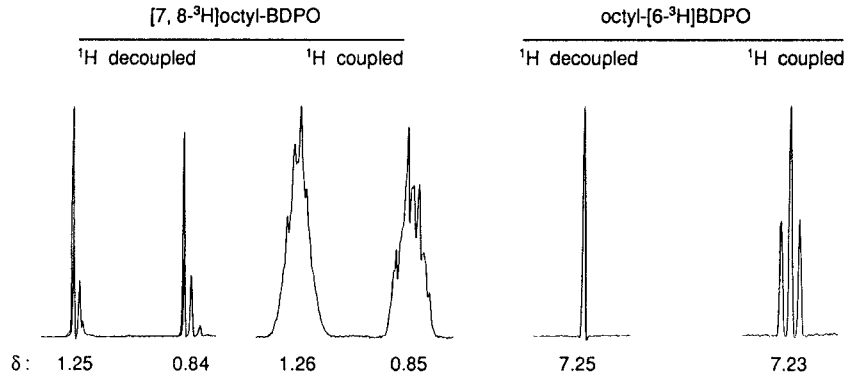


a: PCl_3, THF. b: 1) CH_2=$CH(CH_2)_6MgBr$, Ether, 2) DMSO. c: $^3H_2$, 5% Pt / C, EtOAc, Et_3N.



a: 1) $CH_3(CH_2)_7P(O)Cl_2$, Et_3N, THF. b: $^3H_2$, 10% Pd / C, EtOAc, K_2CO_3.

Two precursors were required. 2-(7,8-Octenyl)-BDPO (3) was prepared by reaction of 2-hydroxybenzyl alcohol (1) with PCl_3 to obtain 2-chloro-4H-1,3,2-benzodioxaphosphorin (2) which was coupled with 7,8-octenylmagnesium bromide followed by oxidation on phosphorus with DMSO. 6-Bromo-2-octyl-BDPO (5) was synthesized by reaction of 5-bromo-2-hydroxybenzyl alcohol (4) and octylphosphonic dichloride. Tritiation of 3 and 5 with $^3H_2$ and suitable catalysts gave [7,8-^{3}H]octyl-BDPO and octyl-[6-^{3}H]BDPO at 38 and 16 Ci/mmol, respectively, with ^3H NMR spectra in acetone-d_6 as follows:



EXPERIMENTAL

^1H and ^{31}P NMR spectra of unlabelled chemicals were determined in $CDCl_3$ (unless otherwise indicated) at 300 and 121.5 MHz, respectively, with a Bruker AM-300 Spectrometer. Chemical shifts are reported as δ referred to tetramethylsilane (^1H and ^3H) and trimethyl phosphate (^{31}P). Mass spectrometry utilized the Hewlett-Packard 5985 system with a direct insertion probe and electron impact (EI, 70 eV, 200°C). HPLC purifications and analyses were performed on a μPorasil column (7.8 mm I.D. x 30 cm, Si-60, 10 μm) with hexane-ethyl acetate (9:1, 5 mL/min), monitoring by UV (254 nm) or (for ^3H compounds) by both UV and radioactivity detectors.

The tritium gas used contained 97.9% $^3H_2$ and 1.76% $^2H^3H$. NMR spectroscopy on ^3H-labelled compounds was carried out on an IBM AF 300 Spectrometer (^3H at 320 MHz, ^1H at 300 MHz) using a $^3H/^1H$ 5-mm dual probe. Specific activities were determined by integration of the ^1H spectra of the ^3H-labelled compounds and total activities by liquid scintillation counting. Radiochemical purity for stored samples was determined before each use by HPLC on a C-18 column (4.0 mm I.D. x 12.5 cm, 80Å) with acetonitrile-water (40 to 100% gradient over 15 min) or by 2D-TLC (silica gel, first ether-benzene 9:1 and then ethyl acetate-hexane 2:1, and radioautography).

Preparation of 2-chloro-4H-1,3,2-benzodioxaphosphorin (2). PCl_3 (4.53 g, 30 mmol) and 1 (3.72 g, 30 mmol) were mixed in dry THF (50 mL) at -60°C and stirred at -60°C to room temperature for 3 h. After evaporation of THF, the residue was distilled in vacuum to give 2 in 76% yield. B.p. 80-82°C/0.9 mm Hg. ^1H NMR: 5.01 (1H, 2d, J=9.6, 14.2 Hz, CH_2O), 5.43 (1H, 2d, J=2.5, 14.3 Hz, CH_2O), 6.95-7.27 (4H, m, aromatic). ^{31}P NMR: 137.6.

Preparation of 2-(7,8-octenyl)-BDPO (3). 8-Bromo-1-octene (1.91 g, 10 mmol) in dry ether (15 mL) was added to Mg turnings (0.24 g, 10 mmol) in dry ether (10 mL). The mixture was heated slowly until Mg totally reacted (30 min) and then cooled to -60°C. One equivalent of 2 was added in dry ether (10 mL) and the reaction warmed gradually to room temperature over 1.5 h. The mixture was cooled again to -60°C and DMSO (1.17 g, 15 mmol) was added and the mixture stirred for another 1.5 h (-60°C to room temperature). After filtration and solvent evaporation, the product was isolated by preparative TLC (silica gel, ether) in 40% yield. ^{1}H NMR : 1.36 [6H, m, C=C-C-$(CH_2)_3$], 1.66 (2H, m, P-C-CH_2), 1.98 (4H, m, P-CH_2, C=C-CH_2), 4.95 (2H, m, C=CH_2), 5.06, 5.46 (2H, m, POCH_2), 5.78 (1H, m, C-CH=C), 7.0-7.4 (4H, m, aromatic). ^{31}P NMR: 25.43. EI-MS(m/z): 108 (base ion), 280 (M^+, 29%). HPLC t_R : 14.8 min.

Preparation of [7,8-^{3}H]octyl-BDPO. A microhydrogenation apparatus was set up with a spoon containing Pt/C (5%) (15 mg) separate from a solution of 3 (25 mg) and dry triethylamine (13 µL) in dry ethyl acetate (3 mL). The entire system was degassed by the application of two freeze-pump-thaw cycles then the catalyst was added and tritium gas was admitted to a pressure of ~740 mm Hg. After the reaction mixture had been stirred at room temperature for 3 h, it was frozen (liquid N_2) and the residual tritium gas pumped away. The apparatus was extensively flushed with N_2 and then methanol (2 mL x 2) was added to the mixture and pumped off to remove any labile or dissolved tritium. Benzene was added, the catalyst filtered off, and the filtrate purified by HPLC. Total activity 1.7 Ci, radiochemical yield 33%, specific activity 38 Ci/mmol. ^{3}H NMR: 0.85 (m, ^{3}H-CH_2), 1.26 (m, ^{3}H-CH), ratio: 1:1.18. ^{1}H NMR: 0.84 (m, ^{3}H-CH_2), 1.27 (m, ^{3}H-CH), 1.44 (2H, m, P-C-C-CH_2), 1.61 (2H, m, P-C-CH_2), 1.99 (2H, m, P-CH_2), 5.34 (2H, m, POCH_2), 7.1-7.4 (4H, m, aromatic). HPLC t_R : 13.3 min.

Preparation of 6-bromo-2-octyl-BDPO (5). Compound 4 (2.0 g, 10 mmol) was mixed with octylphosphonic dichloride (2.2 g, 10 mmol) and triethylamine (2.0 g, 20 mmol) in dry THF (50 mL) at -60°C. The mixture was stirred for 2 h at -60°C to room temperature. After filtration and solvent evaporation, the product was purified by silica gel column chromatography (hexane-ether) to obtain 5 in 42% yield. ^{1}H NMR: 0.85 (3H, t, J=6.5 Hz, CH_3), 1.1-1.4 [10H, m, P-C-C-$(CH_2)_5$], 1.63 (2H, m, P-C-CH_2), 1.98 (2H, m, P-CH_2), 5.02, 5.42 (2H, m, OCH_2), 6.9-7.5 (3H, m, aromatic). ^{31}P NMR: 25.84. EI-MS (m/z): 264 (base ion), 362 (M^+, 27%). HPLC t_R : 10.4 min.

Preparation of octyl-[6-^{3}H]BDPO. Tritiation of 5 (50 mg) was performed in ethyl acetate (4 mL) containing Pd/C (10%) (30 mg) and K_2CO_3 (19 mg) for 3 h using

a technique similar to that for the preparation of [7,8-^{3}H]octyl-BDPO. Total activity 1.8 Ci, radiochemical yield 45%, specific activity 16 Ci/mmol. ^{3}H NMR: 7.23 (t, J=7.47 Hz, aromatic). ^{1}H NMR: 0.87 (3H, t, J=6.03 Hz, CH_3), 1.27 [8H, m, P-C-C-C-$(CH_2)_4$], 1.39 (2H, m, P-C-C-CH_2), 1.61 (2H, m, P-C-CH_2), 1.96 (2H, m, P-CH_2), 5.37 (2H, m, $POCH_2$), 7.1-7.4 (m, aromatic). HPLC t_R: 13.5 min.

Storage stability and enantiomeric resolution of [7,8-^{3}H]octyl-BDPO and octyl-[6-^{3}H]BDPO. The tritium-labelled compounds were of high radiochemical purity (^{3}H NMR spectra shown above; TLC cochromatography) and underwent little decomposition on storage for several months as ethyl acetate solutions in sealed ampoules at -20°C. Rechromatography on a small silica gel column (hexane-ethyl acetate 4 : 1) gave a radiochemical purity of at least 90% based on HPLC with the C-18 column (the remaining portion of the label appeared at the solvent front probably due to hydrolysis as an artifact in the polar HPLC solvent system used). Resolution of the enantiomers can be achieved by HPLC with a CHIRALCEL OC column (hexane-isopropanol 9:1) with *R* eluting before *S*.[14]

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