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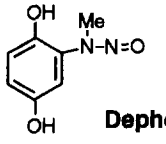
Synthesis and Bioassay of a Protein Tyrosine Phosphatase Inhibitor, Dephostatin

Libing Yu,[1] Andrea McGill,[1] Johnny Ramirez,[1] Peng George Wang,[1]* Zhong-Yin Zhang[2]*

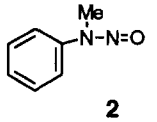
[1]*Department of Chemistry, University of Miami, P.O. Box 249118, Coral Gables, FL 33124.* [2]*Department of Molecular Pharmacology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.*

Abstract: The synthesis of natural product, dephostatin, is described. Both dephostatin and N-methyl-N-nitrosoaniline are found to inactivate the homogeneous recombinant *Yersinia* and mammalian protein tyrosine phosphatases in a concentration and time dependent manner.

The tyrosine phosphorylation on target protein is tightly controlled by the concerted actions of both protein tyrosine kinases and protein tyrosine phosphatases (PTP; EC 2.7.1.112).[1] While much information exists regarding protein tyrosine kinase inhibitors, there has been little progress in the identification and design of PTP inhibitors.[2] Dephostatin **1**, a PTP inhibitor, was recently isolated from the culture broth of *streptomyces sp.*[3] We have been investigating the mechanism of the inhibition by dephostatin since it contains a unique N-nitrosamine functionality. Here we report the synthesis and bioassay of **1** and its unsubstituted precursor, N-methyl-N-nitrosoaniline **2**.



Dephostatin, 1



2

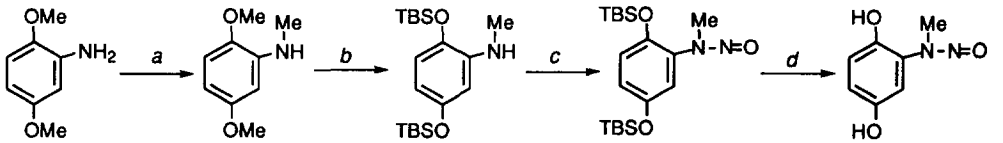
Although the structure of dephostatin seems simple, it turned out difficult to handle since the synthetic intermediates can be easily oxidized. Thus we modified the reported synthetic procedures [4-5] and carried out the synthesis under nitrogen in four steps (**Scheme 1**).

2,5-Dimethoxyaniline **3** (1.7 g, 10 mmol) was first N-methylated with 37% formaldehyde (980 mg, 12 mmol) in the presence of $NaCNBH_3$ (630 mg, 10 mmol) in methanol at 0°C. This reaction was proved to be reliable with a yield around 70% for compound **4**, ^1H NMR (400 MHz, $CDCl_3$) δ 2.83 (3H, NCH_3), 3.76 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 6.15 (1H, m, Ph), 6.20 (1H, m, Ph), 6.63 (1H, m, Ph). The byproduct (2,5-dimethoxyaniline-N, N-dimethylaniline) was readily removed by flash chromatography. The transformation of **4**

to **5** was carried out in a one-pot reaction. At -78° C three equivalents of borontribromide was added to **4** in dichloromethane and the reaction was allowed to warm up to room temperature and kept stirring overnight. The reaction mixture was neutralized with degassed saturated sodium bicarbonate and evaporated *in vacuo* to produce a dry powder. A suspension of the powder in DMF was treated at room temperature with imidazole and chlorotri(n-butyl)silane to afford compound **5**, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70 (4H, m, $2CH_2$), 0.90 (6H, m, $2CH_3$), 1.35 (8H, m, $2CH_2CH_2$), 2.80 (3H, s, NCH_3), 4.05 (1H, br, NH), 6.00 (1H, m, Ph), 6.11 (1H, d, J = 2.4Hz, Ph), 6.52(1H, d, J = 8.4 Hz, Ph). Nitrosation of **5** was carried out by dissolving **5** (540 mg, 1.0 mmol) in 20 mL of tetrahydrofuran at 0°C and adding 3 mL of 1 M hydrochloric acid. Then sodium nitrite (80 mg, 1.1 mmol) was slowly added into the mixture and the reaction was stirred for 5 hours at 0° C. After the reaction was complete, 50 mL of water was added and the solution was extracted with dichloromethane. After removal of solvent, the compound **6** was obtained. Then **6** was dissolved in 20 mL of HF-NaF buffer (pH = 5.0). The desilylation was complete in 30 min. After the solvent was removed the product was purified by silica gel chromatography (eluting with hexane / ethyl acetate, 1 / 1) to afford dephostatin **1** (105 mg), ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 3.36 (3H, s, OMe), 6.82 (2H, m, 2H, Ph), 6.95 (1H, m, Ph), 8.18 (1H, br, OH), 8.40 (1H, br, OH); ^{13}C NMR ($(CD_3)_2CO$) δ 34.4, 113.8, 117.4, 118.5, 131.1, 144.5, 151.5; IR, ν_{max}(cm^{-1}): 3235, 1540, 1475, 1420, 1356, 1275, 1233, 1080, 850, 810, 790, 625.; UV-Vis, λ_{max} (MeOH) 305 nm (ε = 3,200). MS calcd for $C_7H_8N_2O_3H^+$ (M + H$^+$) 169, found 169. The overall yield of the synthesis was about 18%.

N-methyl-N-nitrosoaniline **2** was prepared by the reaction of N-methylaniline with $NaNO_2$ in hydrochloric acid.[6]

Scheme 1. Synthesis of dephostatin **1**



3 **4** **5** **6** **1**

a: HCOH, $NaCNBH_3$, MeOH; *b*: BBr_3, CH_2Cl_2, then TBSCl imidazole/DMF;
c: $NaNO_2$/HCl/THF; *d*: HF-NaF/H_2O/THF

Dephostatin was shown previously to be a competitive inhibitor assayed against a cell membrane preparation containing CD45, a T-cell receptor-like PTP.[3] We tested **1** and **2** against homogeneous recombinant *Yersinia* PTP[7] and mammalian PTP1[7] under comparable conditions. The assay was performed in pH 6.0 buffer containing 50 mM succinate and 1mM EDTA with an ionic strength of 0.15 M. The experiment was started by addition of appropriate concentrations of **1** or **2** to the phosphatase solution. At time intervals, 10 μL aliquots were withdrawn and the enzyme activity was measured by following the production of inorganic phosphate using *p*-nitrophenyl phosphate as a substrate. We found that both PTPs were inactivated by **1** and **2** in a concentration and time dependent manner, but **1** was a much more potent inhibitor than **2** (**Figure 1**). A replot of the apparent

pseudo-first -order rate constants of the inactivation at several inhibitor concentrations (0.5 - 2.8 mM for **1**, and 1 - 25 mM for **2**) indicates that the kinetic order is one with respect to the inhibitor and that there is no evidence of saturation kinetics. This suggests that there is no preequilibrium binding step prior to the inactivation process. The *Yersinia* PTP was inactivated by **1** and **2** with a second-order rate constant of 158 and 0.89 $M^{-1}min^{-1}$, respectively. The rate constants for inactivation of PTP1 by **1** and **2** were 65 and 1.0 $M^{-1}min^{-1}$, respectively. The data indicates that N-nitrosoaniline is the basic functionality for the inhibition and dihydroxyl substitution on the phenyl ring strongly increases the inhibiting ability. Furthermore, the inactivation was prevented by arsenate, a competitive inhibitor of the PTP, suggesting that the modification was towards the enzyme active site. The detailed mechanism of the inhibition by dephostatin against PTP requires further kinetic and structural studies. Such experiments are in progress.

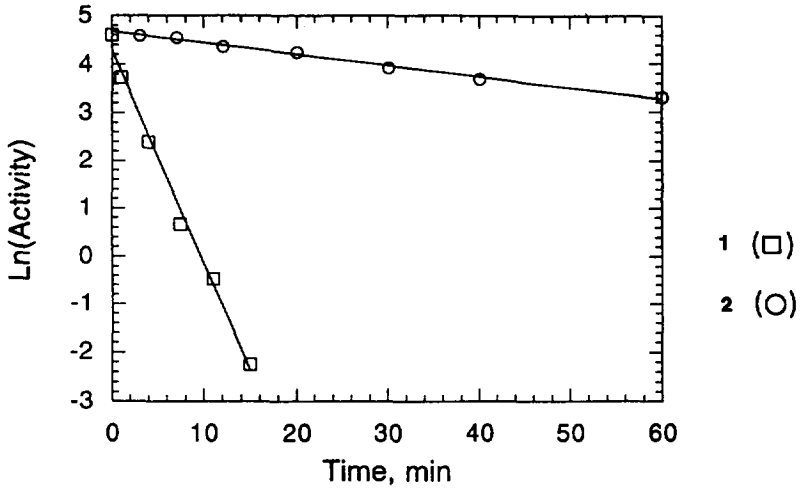


Figure 1. Inactivation of the *Yersinia* PTP by **1** (2.8 mM) or **2** (25 mM).

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