ABSTRACT: We have previously shown that a small peptide bearing the hydrolytically stable phosphotyrosyl (pTyr) mimic, (difluorophosphonomethyl)phenylalanine (F$_2$Pmp), is an extremely potent inhibitor of PTP1B, with an IC$_{50}$ value of 100 nM [Burke, T. R., Kole, H. K., & Roller, P. P. (1994) Biochem. Biophys. Res. Commun. 204, 129–134]. We further demonstrated that removal of the peptide portion and incorporation of the difluorophosphonomethyl moiety onto a naphthalene ring system, but not a phenyl ring system, resulted in good inhibitory potency [Kole, H. K., Smyth, M. S., Russ, P. L., & Burke, T. R., Jr. (1995) Biochem. J. 311, 1025–1031]. In order to understand the structural basis for this inhibition, and to aid in the design of further analogs, we solved the X-ray structure of [1,1-difluoro-1-(2-naphthalenyl)methyl]phosphonic acid (6) complexed within the catalytic site of PTP1B, solved to 2.3 Å resolution. In addition to showing the manner in which the phosphonate group is held within the catalytic site, the X-ray structure also revealed extensive hydrophobic interactions with the naphthalene ring system, beyond that possible with an analog bearing a single phenyl ring. It is further evident that, of the two fluorine atoms, the pro-R α-fluorine interacts with the enzyme to a significantly greater degree than the pro-S α-fluorine, forming a hydrogen bond to Phe 182. On the basis of a computer-assisted molecular modeling analysis, it was determined that addition of a hydroxyl to the naphthyl 4-position, giving [1,1-difluoro-1-[2-(4-hydroxynaphthalenyl)]methyl]phosphonic acid (8), could potentially replace a water molecule situated in the PTP1B·6 complex, thereby allowing new hydrogen-bonding interactions with Lys 120 and Tyr 46. Compound 8 was therefore prepared and found to exhibit a doubling of affinity ($K_i$ = 94 µM) relative to parent unsubstituted 6 ($K_i$ = 179 µM), supporting, in principle, the development of high-affinity ligands based on molecular modeling analysis of the enzyme-bound parent.