Supporting Information

Structure-Based Design and Discovery of Protein Tyrosine Phosphatase Inhibitors Incorporating Novel Isothiazolidinone (IZD) Heterocyclic Phosphotyrosine Mimetics

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Contents:

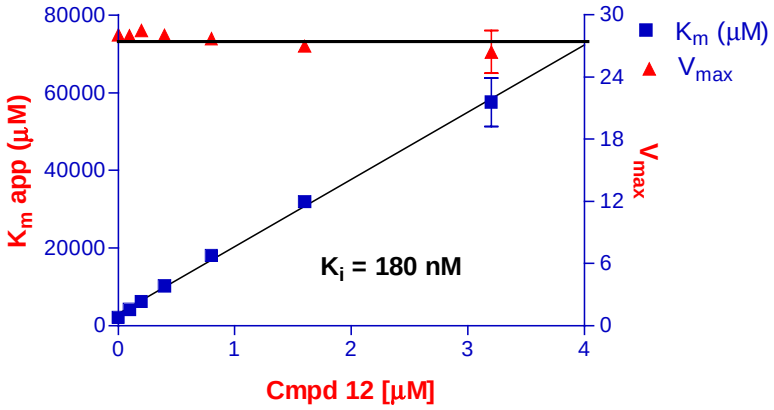
1 - PTP1B Expression and biochemical assays:

Human PTP1B, SHP1, SHP2 and TC-PTP were expressed in E. coli essentially as described in the literature [*J. Biol. Chem.* **2000**, *275 (14)*, 10300-10307; *J. Struct. Biol.* **1997** *120 (2)*, 201-3; *Cell* **1998** *92(4)*, 441–450; *J. Biol. Chem.* **2002** *277(22)*, 19982–19990]. PTP enzymatic assays were performed as described in the literature [*J. Am. Chem. Soc.* **2003**, *125(14)*, 4087-96].

2 - Mode of Inhibition Studies:

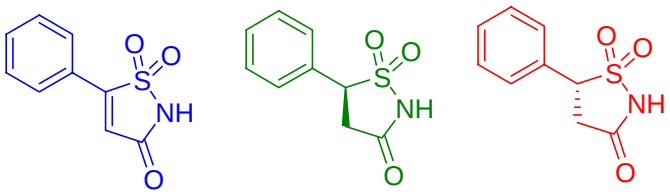
The pNPP assay was performed as described in the literature with the following modifications: Reactions were carried out on 384 well plates containing 80 μL per well. Dilutions of compound were made in glass tubes in DMSO, and PTP1B in assay buffer (25 mM Bis-Tris-Propane, pH 7.0, 1 mM EDTA, 0.1 mg/mL BSA) was added (final [DMSO] of 2%). To 60 μL of this mixture, 20 μL of 4-nitrophenyl phosphate (pNPP) was added per well, for final pNPP concentrations between 0.46 mM and 30 mM

and final [PTP1B] of 5 nM. Rate of formation of the phenolate ion was monitored at 410 nm on a spectramax 384 plate reader. Slopes of initial reaction rates (15 minute reactions) were plotted and fit to the Michaelis-Mention equation by nonlinear regression analysis (Graphpad Prism). A competitive K_i was determined by linear regression of a plot of Km vs. [inhibitor], such that K_i = -(x-intercept). Results for compound **12** are shown below graphically.



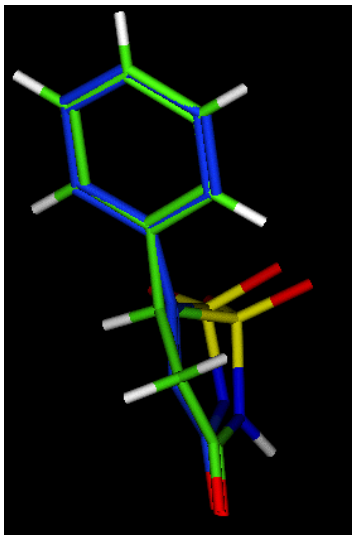
3 –Ab Initio Modeling – Rationale for *S*-IZD preference

Energy profiles for rotation of the bond joining aryl and IZD rings were calculated for the simple aryl unsaturated IZD and the aryl *R*- and *S*-IZD systems, using the program SPARTAN (Spartan'04, Wavefunction, Inc., Irvine, CA) and a 6-31G* basis set. Minima were compared with the crystal structure of an oxidized IZD inhibitor (at that time the only solved structure) by superposition of the aromatic rings (shown below). At the minimum energy conformation of S-IZD, all four hydrogen bond acceptors are within 0.5 Å of their unsaturated IZD analogs in the crystal structure of the complex.

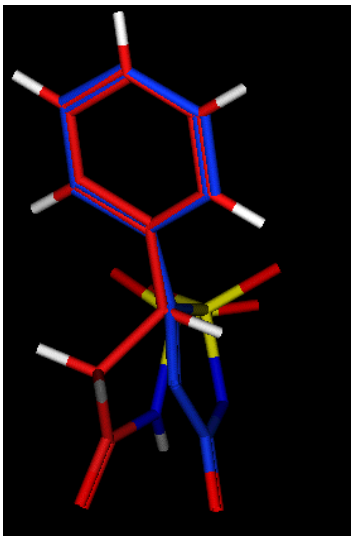


Unsat. IZD ***S*-IZD** ***R*-IZD**

S-IZD overlay Unsat. IZD (Blue-X-ray)



R-IZD overlay with Unsat. IZD (Blue-X-ray)



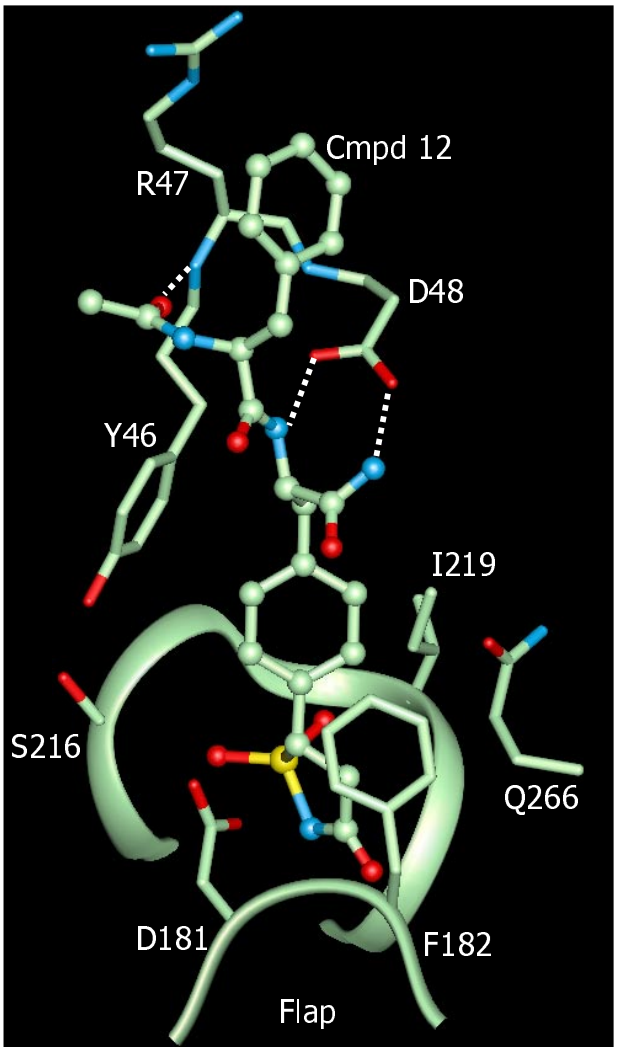
We then postulated that because of the 8 hydrogen bond donors of the active site, acceptors of the unsat. IZD heterocycle and sat. IZD heterocycle would be positioned identically when bound to the protein. We found that although crystallographic unsaturated IZD was not at its energy minimum, a bond rotation of about 30° from the minimum would bring all four acceptors within 0.5 Å of their crystal positions. The rotation corresponds to an energy loss of almost 1 kcal/Mol, about an order of magnitude difference in affinity. It is not possible to rotate the *R*-IZD to bind in an analogous manner, consistent with its weak binding.

We conjectured that the *S*-IZD would be more effective than the unsat. IZD because the *S*-IZD would bind in its lowest energy form, whereas the calculations suggest the unsat. IZD binds at a cost of about 1 kcal/Mol. The crystal structure of inhibitor **12** in Figure 5, as well as, the relative IC_{50}'s of the unsat. IZD inhibitor **8** and the sat. *S*-IZD inhibitor **12** are in accord with our binding model, consistent with this hypothesis. A full description of both crystallographic and theoretical results will be presented in a subsequent paper.

4-Crystallography:

Crystals of PTP1B (residues 1-321) in complex with **12** were grown using the vapor diffusion method by mixing equal volumes of protein-inhibitor (10 mg/ml protein and 3 mM inhibitor) and well solutions (100 mM Hepes pH 6.6, 14-16% PEG 8000, and 200 mM Magnesium Acetate) at 4 ºC. Diffraction data were collected at -180 ºC with a Rigaku/MSC RAXIS IV ++ imaging system mounted on a Rigaku/MSC MicroMax^{TM}-007 rotating anode microfocus generator (CuK_{α}). Intensities were integrated and scaled with CrystalClear (Rigaku/MSC). The complex crystallized in the space group $P2_1$ with one molecule in the asymmetric unit and the following unit cell parameters: a = 43.9 Å, b = 88.4 Å, c = 49.8 Å and β = 96.8º. The structure was solved by molecular replacement using CNX (Accelrys). The starting model was derived from the atomic coordinates of PTP1B in complex with a peptide substrate (Protein Data Bank entry code 1EE0). Rotation and translation searches yielded single consistent solutions for data between 15-4 Å. The conformation of **12** was unambiguously determined after several cycles of refinement using CNX (Accelrys). Solvent molecules were added by visual inspection of electron density maps using X-SOLVATE (Accelrys). Refinement reduced the starting

R_{factor} of ~45% to 19.9% (R_{free} = 25.4%) for data in the resolution range 8-2.1 Å. Full view of crystal structure of PTP1B/**12** shown below.

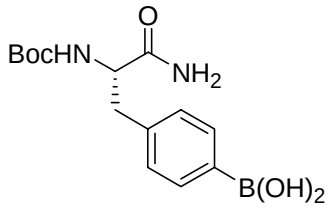


5– Synthetic Experimentals:

All reactions were run under an atmosphere of dry nitrogen. All solvents were used without further purification as acquired from commercial sources. NMR spectra were obtained using either a Varian Mercury-300, Mercury-400, or Inova-500 spectrometer. Chemical shifts are reported in parts per million relative to

tetramethylsilane (TMS) as internal standard. All final products, compounds **8**, **11**, **12**, **13**, were characterized by ^{1}H NMR, ^{13}C NMR, HRMS, LCMS, and two HPLC methods.

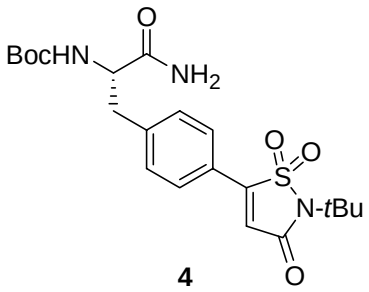
Purifications by flash chromatography were performed on RediSep columns using an Isco CombiFlash SG100c. Preparative LCMS purifications were performed on a Waters FractionLynx system using mass directed fractionation and compound-specific method optimization [*J. Comb. Chem.* **2004**, *6*, 874-883]. The LC method utilized a Waters SunFire column (19 x 100 mm, 5 μM particle size), with a water/0.1% TFA and acetonitrile/0.1% TFA gradient at a flow rate of 30 mL/min over a total run time of 5 min.



2

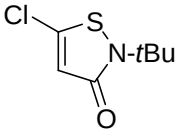
2: (4-(2*S*)-3-amino-2-[(tert-butoxycarbonyl)amino]-3-oxopropylphenyl)boronic acid. A solution of *N*-(*tert*-butoxycarbonyl)-4-(dihydroxyboryl)-l-phenylalanine (5.11 g, 16.5 mmol) in 1,4-dioxane (80 mL) was treated with *N*,*N*-dimethylformamide (14 mL), pyridine (4.0 mL, 50 mmol), di-*tert*-butyldicarbonate (4.69 g, 21.5 mmol) and ammonium bicarbonate (2.61 g, 33.0 mmol). The reaction mixture was stirred at 25 °C for 60 h. The light suspension was diluted with ethyl acetate (650 mL) and washed with 0.1 N hydrochloric acid (3 x 150 mL). The combined aqueous layers were re-extracted with ethyl acetate (2 x 100 mL). The combined organic layers were washed with brine (2 x 100 mL), dried with sodium sulfate, filtered, and concentrated to give **2** (5.20 g, 97%) as a white solid. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.96 (s, 2 H), 7.69 (d, *J* = 7.8 Hz, 2 H), 7.38 (br s, 1 H), 7.22 (d, *J* = 7.8 Hz, 2 H), 7.02 (br s, 1 H), 6.82 (d, *J* = 8.8 Hz, 1 H),

4.11 - 4.07 (m, 1 H), 2.95 (dd, *J* = 13.7, 4.4 Hz, 1 H), 2.76 - 2.71 (m, 1 H), 1.31 (s, 9 H); LCMS calculated for $C_{14}H_{21}BN_2O_5Na$ $(M+Na)^+$: m/z = 331.0.

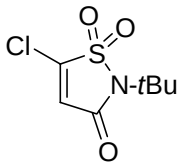


4

4: *tert*-Butyl (1*S*)-2-amino-1-[4-(2-tert-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethylcarbamate.



Step 1: 2-*tert*-Butyl-5-chloro-isothiazol-3-one. A solution of *N*-*tert*-butyl-3-(2-*tert*-butylcarbamoyl-ethyldisulfanyl)-propionamide [*J. Med. Chem.* **1989**, *32*, 1024-1033] (20.0 g, 0.062 mol) in 1,2-dichloroethane (313 mL) was treated with sulfuryl chloride (15.2 mL, 0.187 mol) dropwise at 0 °C and stirred at 25 °C for 3 h. The reaction mixture was cooled to 0 °C, quenched with water (250 mL), and stirred for 10 min. The organic layer was separated and the aqueous layer was re-extracted with dichloromethane (250 mL). The combined organic layers were dried with magnesium sulfate, filtered, and concentrated to a light brown oil. The crude oil was purified by flash column chromatography (100% hexane to 40% ethyl acetate/hexane to 100% ethyl acetate) to yield 2-*tert*-butyl-5-chloro-isothiazol-3-one (5.04 g, 42%) as an off-white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.13 (s, 1 H), 1.55 (s, 9 H).

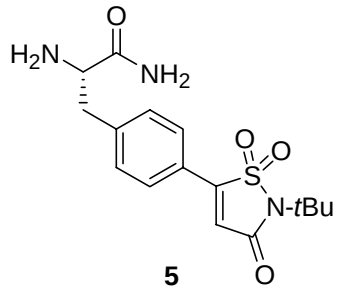


3

Step 2: 2-*tert*-Butyl-5-chloro-1,1-dioxo-1,2-dihydro-1λ^6-isothiazol-3-one. A solution of 2-tert-butyl-5-chloro-isothiazol-3-one (18.7 g, 98 mmol) in dichloromethane (488 mL) was treated with *m*-chloroperbenzoic acid (109 g, 0.488 mol) portion-wise at 0 °C and stirred at 25 °C for 3 d. The reaction mixture was cooled to 0 °C and 10% sodium bisulfite (400 mL) was added slowly via an additional funnel. The reaction mixture was extracted with ethyl acetate (1 L) and the organic layer was separated and washed with 10% sodium bisulfite (2 x 500 mL), saturated sodium bicarbonate (5 x 300 mL), and brine (500 mL) and was dried with magnesium sulfate, filtered, and concentrated to give a white solid. The crude residue was purified by flash column chromatography (100% hexane to 10% ethyl acetaet/hexane to 20%) to yield **3** (8.7 g, 40%) as an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.56 (s, 1 H), 1.69 (s, 9 H).

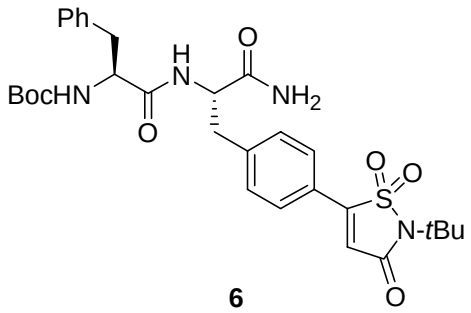
Step 3: *tert*-Butyl (1*S*)-2-amino-1-[4-(2-tert-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethylcarbamate. A solution of (4-(2*S*)-3-amino-2-[(tert-butoxycarbonyl)amino]-3-oxopropylphenyl)boronic acid (4.00 g, 13.0 mmol), **3** (3.48 g, 15.6 mmol), [1,1'-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (1:1) (1.80 g, 2.21 mmol), and potassium carbonate (8.97 g, 64.9 mmol) in 1,4-dioxane (65 mL) was degassed by the freeze-thaw method thrice. The reaction mixture was heated at 80 °C for 16 hours, cooled to 25 °C, poured into water (250 mL), and extracted with dichloromethane (4 x 100 mL). The combined

organic layers were washed with brine (150 mL), dried with sodium sulfate, filtered, and concentrated *in vacuo*. The crude residue was purified by flash column chromatography (100% dichloromethane to 10% methanol/dichloromethane) to yield **4** (4.56 g, 78%) as a red solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, *J* = 8.2 Hz, 2 H), 7.39 (d, *J* = 8.4 Hz, 2 H), 6.63 (s, 1 H), 5.95 (br s, 1 H), 5.44 (br s, 1 H), 5.09 - 5.00 (m, 1 H), 4.45 - 4.39 (m, 1 H), 3.19 (dd, *J* = 13.9, 6.8 Hz, 1 H), 3.15 - 3.05 (m, 1 H), 1.73 (s, 9 H), 1.41 (s, 9 H); LCMS calculated for $C_{21}H_{29}N_3O_6SNa$ $(M+Na)^+$: m/z = 474.0.



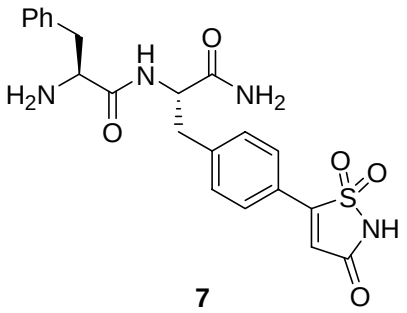
5

5: 4-(2-tert-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)-L-phenylalaninamide hydrochloride. A solution of tert-butyl **4** (4.76 g, 10.5 mmol) in dichloromethane (15.6 mL) was treated with 4 M of hydrogen chloride in 1,4-dioxane (37 mL). The reaction mixture was stirred at 25 °C for 1.5 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (2 x 50 mL) to yield **5** (4.23 g, 98%) as a white solid. ^{1}H NMR (400 MHz, CD_3OD): δ 7.89 (d, *J* = 8.4 Hz, 2 H), 7.52 (d, *J* = 8.4 Hz, 2 H), 7.07 (s, 1 H), 4.16 (dd, *J* = 8.2, 6.2 Hz, 1 H), 3.35-3.30 (m, 1 H), 3.15 (dd, *J* = 14.3, 8.2 Hz, 1 H), 1.70 (s, 9 H); LCMS calculated for $C_{16}H_{22}N_3O_4S$ $(M+H)^+$: m/z = 352.1.



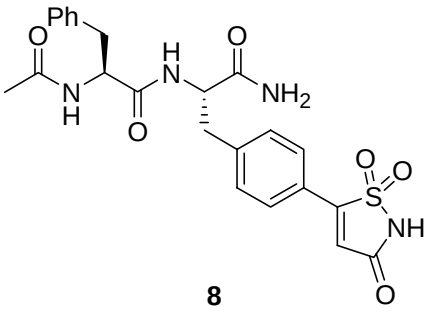
6

6: *N*-(tert-butoxycarbonyl)-L-phenylalanyl-4-(2-tert-butyl-1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)-L-phenylalaninamide. A solution of **5** (940 mg, 2.42 mmol) and *N*-(tert-butoxycarbonyl)-l-phenylalanine (772 mg, 2.91 mmol), in dichloromethane (10 mL) and *N*,*N*-dimethylformamide (2 mL), was treated with *N*,*N*-diisopropylethylamine (1 mL, 6 mmol), 2.0 M of 1-hydroxy-7-azabenzotriazole in *N*,*N*-dimethylformamide (242 μL), and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (697 mg, 3.64 mmol). The reaction mixture was stirred at 25 °C for 16 h, diluted with ethyl acetate (200 mL), and washed with 0.1 N HCl (2 x 100 mL), saturated sodium bicarbonate (2 x 100 mL), and brine (100 mL), dried with sodium sulfate, filtered, and concentrated *in vacuo*. The crude residue was recrystallized from ethyl acetate/hexane to yield **6** (1.23 g, 84%) as a pale beige solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.67 (d, *J* = 8.3 Hz, 2 H), 7.34 - 7.28 (m, 3 H), 7.23 (d, *J* = 7.8 Hz, 2 H), 7.18 - 7.16 (m, 2 H), 6.61 (s, 1 H), 6.35 (br s, 1 H), 6.15 (br s, 1 H), 5.32 (br s, 1 H), 4.89 (d, *J* = 5.9 Hz, 1 H), 4.77 - 4.73 (m, 1 H), 4.26 (dt, *J* = 6.3, 6.3 Hz, 1 H), 3.22 - 3.19 (m, 1 H), 3.07 - 2.97 (m, 3 H), 1.73 (s, 9 H), 1.33 (s, 9 H); LCMS calculated for $C_{25}H_{31}N_4O_5S$ ([M-Boc]+H)$^+$: m/z = 499.2.



7

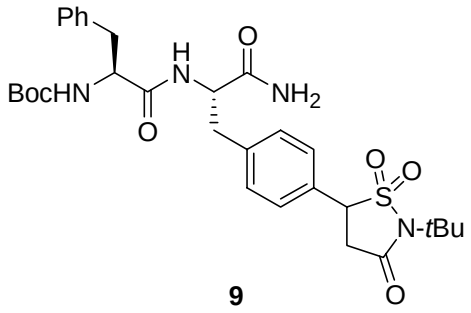
7: (2*S*)-2-amino-*N*-(1*S*)-2-amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl-3-phenylpropanamide trifluoroacetate. A solution of **6** (500 mg, 840 μmol) in trifluoroacetic acid (8 mL, 100 mmol) was treated with triisopropylsilane (240 μL, 1.2 mmol). The reaction mixture was heated at 70 °C for 16 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (2 x 50 mL). The crude residue was purified by preperative LCMS (acetonitrile/water/TFA) to yield **7** (300 mg, 65%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.85 (dd, *J* = 6.8, 2.0 Hz, 2 H), 7.49 (d, *J* = 8.3 Hz, 2 H), 7.39 - 7.36 (m, 2 H), 7.34 - 7.30 (m, 3 H), 6.92 (s, 1 H), 4.76 (dd, *J* = 9.3, 5.9 Hz, 1 H), 4.10 (dd, *J* = 8.3, 4.9 Hz, 1 H), 3.32 - 3.24 (m, 2 H), 3.09 - 3.00 (m, 2 H); LCMS calculated for $C_{21}H_{23}N_4O_5S$ $(M+H)^+$: m/z = 443.1.



8

8: (2*S*)-2-(acetylamino)-*N*-(1*S*)-2-amino-1-[4-(1,1-dioxido-3-oxo-2,3-dihydroisothiazol-5-yl)benzyl]-2-oxoethyl-3-phenylpropanamide. A solution of **7** (30 mg, 54 μmol) and acetic acid (9.3 μL, 160 μmol) in dichloromethane (600 μL, 9.4 mmol)

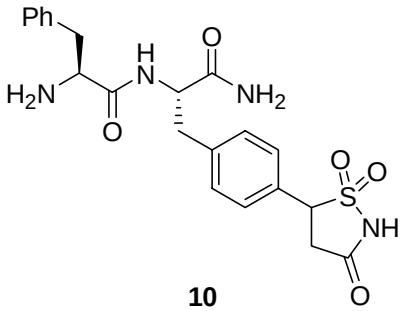
and *N*,*N*-dimethylformamide (180 μL, 2.3 mmol) was treated with *N*,*N*-diisopropylethylamine (31 μL, 180 μmol), 2.0 M of 1-hydroxy-7-azabenzotriazole in *N*,*N*-dimethylformamide (5.5 μL, 11 μmol), and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (25.8 mg, 135 μmol). The reaction mixture was stirred at 25 °C for 60 h, concentrated *in vacuo*, and purified by preperative LCMS (acetonitrile/water/TFA) to yield **8** (19.5 mg, 75%). ^{1}H NMR (500 MHz, CD_3OD): δ 8.20 (d, *J* = 8.3 Hz, 0.25 H), 7.85 (d, *J* = 8.3 Hz, 2 H), 7.48 (d, *J* = 8.8 Hz, 2 H), 7.31 - 7.28 (m, 2 H), 7.25 - 7.22 (m, 3 H), 7.09 (s, 1 H), 4.77 - 4.64 (m, 1 H), 4.60 (dd, *J* = 8.8, 5.9 Hz, 1 H), 3.29 (dd, *J* = 13.7, 4.9 Hz, 1 H), 3.08 - 2.98 (m, 2 H), 2.84 (dd, *J* = 13.7, 8.8 Hz, 1 H), 1.89 (s, 3 H); ^{13}C NMR (125 MHz, 1:1 CD_3CN:CD_3OD): δ 174.6, 173.0, 172.9, 164.6, 154.4, 143.7, 138.5, 131.7, 130.1, 129.3, 129.2, 127.8, 124.9, 121.0, 55.9, 55.9, 55.0, 38.3, 22.6; HRMS calculated for $C_{23}H_{25}N_4O_6S$ $(M+H)^+$: m/z = 485.1485.



9

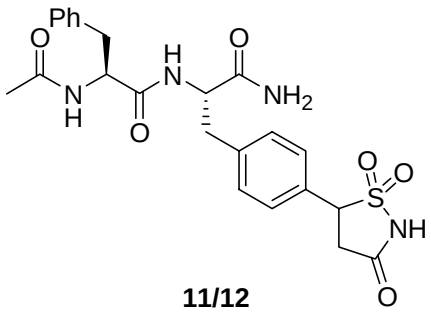
9: tert-butyl [(1*S*)-2-((1*S*)-2-amino-1-[4-(2-tert-butyl-1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethylamino)-1-benzyl-2-oxoethyl]carbamate. A degassed solution of **6** (700 mg, 1.17 mmol) in methanol (15 mL, 370 mmol) was treated with palladium (100 mg, 940 μmol). The reaction mixture was treated with hydrogen at 50 psi in a Parr shaker for 16 h, filtered, and concentrated *in vacuo* to yield **9** (682 mg,

96%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31 - 7.27 (m, 5 H), 7.26 - 7.20 (m, 2 H), 7.19 - 7.16 (m, 2 H), 5.34 (s, 1 H), 4.77 (dt, *J* = 9.0, 1.6 Hz, 1 H), 4.66 - 4.61 (m, 1 H), 4.30 - 4.22 (m, 1 H), 3.44 - 3.00 (m, 6 H), 2.88 - 2.85 (m, 1 H), 2.73 (s, 2 H), 1.65 (s, 9 H), 1.37 (s, 9 H); LCMS calculated for $C_{25}H_{33}N_4O_5S$ ($[M\text{-}Boc]+H)^+$: m/z = 501.2.



10

10: (2*S*)-2-amino-*N*-(1*S*)-2-amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl-3-phenylpropanamide trifluoroacetate. A solution of **9** (682 mg, 1.14 mmol) in trifluoroacetic acid (11 mL, 141 mmol) was treated with triisopropylsilane (326 μL, 1.59 mmol). The reaction mixture was heated at 70 °C for 16 h. The reaction mixture was concentrated *in vacuo* and co-evaporated with toluene (2 x 50 mL). The crude residue was purified by preperative LCMS (acetonitrile/water/TFA) to yield **10** (502 mg, 79%) as a white solid. ^{1}H NMR (500 MHz, CD_3OD): δ 7.47 - 7.41 (m, 2 H), 7.39 - 7.37 (m, 4 H), 7.34 - 7.30 (m, 3 H), 5.28 - 5.14 (m, 1 H), 4.72 (dd, *J* = 8.8, 5.9 Hz, 1 H), 4.10 - 4.00 (m, 1 H), 3.39 - 3.35 (m, 1 H), 3.32 - 3.28 (m, 1 H), 3.24 - 3.20 (m, 1 H), 3.05 - 2.99 (m, 2 H); LCMS calculated for $C_{21}H_{25}N_4O_5S$ $(M+H)^+$: m/z = 445.1.

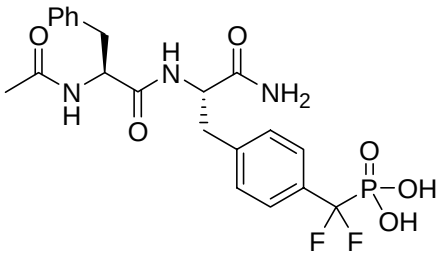


11/12

11/12: (2*S*)-2-(acetylamino)-*N*-(1*S*)-2-amino-1-[4-(1,1-dioxido-3-oxoisothiazolidin-5-yl)benzyl]-2-oxoethyl-3-phenylpropanamide. A solution of **10** (113 mg, 202 μmol) and acetic acid (34 μL, 607 μmol) in dichloromethane (2 mL, 31.2 mmol) and *N*,*N*-dimethylformamide (1 mL, 12.9 mmol) was treated with *N*,*N*-diisopropylethylamine (88 μL, 506 μmol), 0.6 M of 1-hydroxy-7-azabenzotriazole in *N*,*N*-dimethylformamide (67 μL, 40.5 μmol), and *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (58.2 mg, 303 μmol). The reaction mixture was stirred at 25 °C for 16 h. The reaction mixture was concentrated *in vacuo* and purified by preparative LCMS (acetonitrile/water/TFA) to yield the diastereomeric mixture of **11** and **12** (81 mg, 82%) as a white solid. Diastereomeric mixture of **11** and **12**: ^{1}H NMR (500 MHz, CD_3OD): δ 7.37 (d, *J* = 7.8 Hz, 2 H), 7.30 - 7.25 (m, 4 H), 7.22 - 7.19 (m, 3 H), 4.88 - 4.83 (m, 1 H), 4.63 - 4.56 (m, 2 H), 3.27 (dd, *J* = 17.1, 8.8 Hz, 1 H), 3.22 - 3.12 (m, 3 H), 3.07 (dd, *J* = 14.0, 5.7 Hz, 1 H), 2.99 - 2.94 (m, 1 H), 2.82 (dd, *J* = 13.5, 8.8 Hz, 1 H), 1.90 (s, 3 H); ^{13}C NMR (125 MHz, CD_3OD, 30 °C): δ 177.4, 175.7, 173.6, 173.5, 139.3, 138.6, 132.3, 130.9, 130.4, 130.0, 129.6, 127.9, 67.0, 56.5, 55.5, 41.8, 38.5, 38.5, 22.6; HRMS calculated for $C_{23}H_{27}N_4O_6S$ $(M+H)^+$: m/z = 487.1675. A solution of the diastereomeric mixture of **11** and **12** (80 mg, 0.16 mmol) and *N*,*N*-diisopropylethylamine (43 μL, 0.25 mmol) in acetonitrile (6.7 mL) was treated with 2-(trimethylsilyl)ethoxymethyl chloride

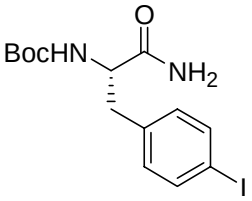
(35 μL, 0.20 mmol). The reaction mixture was heated at reflux for 1 h, cooled to 25 °C and diluted with ethyl acetate and 0.1 N HCl. The organic layer was separated and washed with brine, dried with sodium sulfate, filtered, and concentrated *in vacuo*. The crude solid was recrystallized with ethyl acetate/hexane to yield the desired SEM-protected product which was purified by normal phase chiral HPLC (ChiralCel OJ-H [20 x 250 mm, 5 μm], 15% ethanol/15% methanol/70% hexane, 15 mL/min, 30 °C) to yield SEM-**11** (peak 1) (38 mg, 42%) and SEM-**12** (peak 2) (38 mg, 42%). A solution of SEM-**11** (38 mg, 62 μmol) in dichloromethane (2 mL) was treated with trifluoroacetic acid (1 mL) at 25 °C for 15 min. The reaction mixture was concentrated *in vacuo* and purified by preparative LCMS (acetonitrile/water/TFA) to yield **11** (peak 1) (17 mg, 57%) as a white solid. Compound **12** (peak 2) (15 mg, 50%) was prepared in a similar manner. **11** (Peak 1): ^{1}H NMR (500 MHz, CD_3CN, 30 °C): δ 7.42 (d, *J* = 8.1 Hz, 2 H), 7.34 (d, *J* = 8.4 Hz, 2 H), 7.29 - 7.25 (m, 2 H), 7.24 - 7.21 (m, 1 H), 7.18 - 7.15 (m, 2 H), 6.94 (d, *J* = 8.4 Hz, 1 H), 6.62 (d, *J* = 7.0 Hz, 1 H), 6.34 (br s, 1 H), 5.74 (br s, 1 H), 5.09 (dd, *J* = 8.7, 8.7 Hz, 1 H), 4.58 - 4.50 (m, 1 H), 4.42 - 4.37 (m, 1 H), 3.30 (dd, *J* = 9.7, 8.4 Hz, 2 H), 3.24 (dd, *J* = 14.1, 4.7 Hz, 1 H), 3.00 (dd, *J* = 14.1, 5.7 Hz, 1 H), 2.94 (dd, *J* = 14.1, 9.1 Hz, 1 H), 2.76 (dd, *J* = 14.1, 9.1 Hz, 1 H), 1.83 (s, 3 H); ^{13}C NMR (125 MHz, CD_3CN, 30 °C): δ 173.6, 172.1, 171.7, 168.4, 140.5, 138.4, 131.1, 130.2, 130.0, 129.4, 128.4, 127.4, 66.0, 56.0, 54.6, 38.3, 37.8, 37.7, 22.8; HRMS calculated for $C_{23}H_{27}N_4O_6S$ $(M+H)^+$: m/z = 487.1675. **12** (Peak 2): ^{1}H NMR (500 MHz, CD_3CN + 10 μL TFA): δ 7.62 (d, *J* = 8.3 Hz, 1 H), 7.48 (d, *J* = 7.3 Hz, 1 H), 7.40 (d, *J* = 8.3 Hz, 2 H), 7.34 (d, *J* = 8.3 Hz, 2 H), 7.29 - 7.27 (m, 2 H), 7.23 - 7.19 (m, 3 H), 6.87 (br s, 1 H), 6.44 (br s, 1 H), 5.09 (dd, *J* = 8.8, 8.8 Hz, 1 H), 4.54 - 4.50 (m, 1 H), 4.48 - 4.43 (m, 1 H), 3.33 (dd, *J*

= 17.6, 9.8 Hz, 1 H), 3.26 (dd, *J* = 17.1, 8.3 Hz, 1 H), 3.19 (dd, *J* = 14.2, 4.9 Hz, 1 H), 2.97 (dd, *J* = 14.2, 5.4 Hz, 1 H), 2.93 (dd, *J* = 14.2, 9.3 Hz, 1 H), 2.75 (dd, *J* = 14.2, 9.3 Hz, 1 H), 1.81 (s, 3 H); ^{13}C NMR (125 MHz, CD_3CN, 30 °C): δ 173.5, 172.0, 171.6, 168.4, 140.5, 138.4, 130.9, 130.1, 129.9, 129.3, 128.2, 127.4, 65.9, 56.0, 54.4, 38.2, 37.7, 37.5, 22.8; HRMS calculated for $C_{23}H_{27}N_4O_6S$ $(M+H)^+$: m/z = 487.1669.



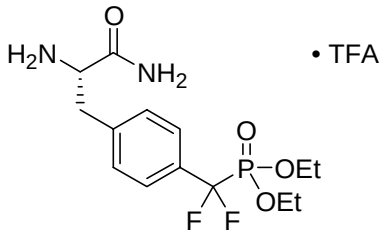
13

13: [[4-((2*S*)-2-[(2*S*)-2-(acetylamino)-3-phenylpropanoyl]amino-3-amino-3-oxopropyl)phenyl](difluoro)methyl]phosphonic acid.

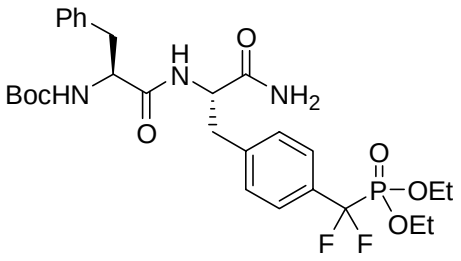


Step 1: [(*S*)-1-Carbamoyl-2-(4-iodo-phenyl)-ethyl]-carbamic acid tert-butyl ester. A solution of boc-phe(4-I)-OH (5.62 g, 14.4 mmol) in 1,4-dioxane (25 mL) was treated with pyridine (0.75 mL), di-*tert*-butyldicarbonate (4.71 g, 21.6 mmol), and ammonium bicarbonate (1.71 g, 21.6 mmol), and stirred for 16 hrs. The reaction mixture was quenched with water and the white precipitate was filtered. The solid was washed with water and dried *in vacuo* to give [(*S*)-1-carbamoyl-2-(4-iodo-phenyl)-ethyl]-carbamic acid tert-butyl ester (5.56 g, 99%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.63 (d, *J* = 9.0 Hz, 2 H), 6.99 (d, *J* = 9.0 Hz, 2 H), 5.80 (br s, 1 H), 5.38 (br s, 1 H), 5.00 (m, 1 H), 4.33 (m, 1

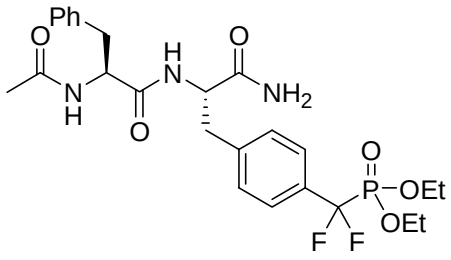
H), 3.02 (d, *J* = 6.8 Hz, 2 H), 1.42 (s, 9 H); LCMS calculated for $C_{14}H_{20}IN_2O_3$ $(M+H)^+$: m/z = 391.



Step 2: [4-((*S*)-2-Amino-2-carbamoyl-ethyl)-phenyl]-difluoro-methyl-phosphonic acid diethyl ester, trifluoro acetate. A solution of freshly prepared diethyl(bromocadmiumdifluoro)phosphonate [*Tetrahedron Lett.* **1996**, *37*, 2745-2748; *Tetrahedron* **1997**, *53*, 11171-11178] (48.2 mL, 73 mmol in 70 mL DMF) in a flame-dried round bottomed flask under a nitrogen atmosphere was with CuCl (5.78 g, 58.4 mmol), [(*S*)-1-carbamoyl-2-(4-iodo-phenyl)-ethyl]-carbamic acid tert-butyl ester (5.56 g, 14.3 mmol), and DMF (25 mL). The reaction mixture was stirred at room temperature for 5 d and quenched with saturated ammonium chloride solution and extracted with EtOAc. The organic extracts were washed with water and brine, dried over sodium sulfate, filtered, and concentrated *in vacuo*. The crude residue was treated with TFA/DCM (1:1) for 30 min. The volatiles were removed *in vacuo* and purified by preparative LCMS (acetonitrile/water/TFA) to afford [4-((*S*)-2-amino-2-carbamoyl-ethyl)-phenyl]-difluoro-methyl-phosphonic acid diethyl ester (3.29 g, 50%). LCMS calculated for $C_{14}H_{22}F_2N_2O_4P$ $(M+H)^+$: m/z = 351.



Step 3: Diethyl [4-[(2*S*)-3-amino-2-((2S)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropanoylamino)-3-oxopropyl]phenyl(difluoro)methyl]phosphonate. A solution of [4-((*S*)-2-amino-2-carbamoyl-ethyl)-phenyl]-difluoro-methyl-phosphonic acid diethyl ester (92.0 mg, 0.20 mmol) in dichloromethane (1.0 mL) and DMF (1.0 mL), was treated with *N*,*N*-diisopropylethylamine (86 μL, 0.50 mmol), 0.6 M of 1-hydroxy-7-azabenzotriazole in DMF (70 μL), *N*-(tert-butoxycarbonyl)-L-phenylalanine (160 mg, 0.59 mmol), and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (57 mg, 0.30 mmol) and stirred overnight at 25 °C. The reaction mixture was concentrated *in vacuo* and the crude residue was purified by preparative LCMS (acetonitrile/water/TFA) to afford diethyl [4-[(2*S*)-3-amino-2-((2*S*)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropanoylamino)-3-oxopropyl]phenyl(difluoro)methyl]phosphonate (60 mg, 51%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.92 (d, *J* = 8.5 Hz, 1 H), 7.46 - 7.36 (m, 4 H), 7.25 - 7.12 (m, 5 H), 6.95 (d, *J* = 8.6 Hz, 1 H), 4.60 - 4.40 (m, 1 H), 4.15 - 3.95 (m, 5 H), 3.06 (dd, *J* = 13.4 Hz, 1 H), 2.92 - 2.78 (m, 2 H), 2.68-2.58 (m, 1 H), 1.22 (s, 9 H), 1.16 (t, *J* = 7.4 Hz, 6 H); LCMS calculated for $C_{28}H_{39}F_2N_3O_7P$ $(M+H)^+$: m/z = 598.



Step 4: Diethyl [[4-((2S)-2-[(2S)-2-(acetylamino)-3-phenylpropanoyl]amino-3-amino-3-oxopropyl)phenyl](difluoro)methyl]phosphonate. A solution of diethyl [4-[(2*S*)-3-amino-2-((2*S*)-2-[(tert-butoxycarbonyl)amino]-3-phenylpropanoylamino)-3-oxopropyl]phenyl(difluoro)methyl]phosphonate (72 mg, 0.12 mmol) in dichloromethane (1.0 mL) was treated with trifluoroacetic acid (1.0 mL) and stirred for 15 min. The reaction mixture was concentrated *in vacuo* and the residue was treated with pyridine (0.5 mL) and acetic anhydride (0.5 mL) and stirred at 30 °C for 30 min. The reaction mixture was concentrated *in vacuo* and the crude residue was purified on the preparative LCMS (acetonitrile/water/TFA) to afford diethyl [[4-((2*S*)-2-[(2*S*)-2-(acetylamino)-3-phenylpropanoyl]amino-3-amino-3-oxopropyl)phenyl](difluoro)methyl]phosphonate (63 mg, 96%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.07 (m, 2 H), 7.45 (m, 2 H), 7.38 (m, 2 H), 7.32 (br s, 1 H), 7.25 - 7.10 (m, 7 H), 4.42 (m, 2 H), 4.04 (m, 4 H), 3.07 (dd, *J* = 14.0, 4.8 Hz, 1 H), 2.88 (m, 2 H), 2.64 (dd, *J* = 14.0, 10.1 Hz, 1 H), 1.72 (s, 3 H), 1.18 (dt, *J* = 7.2, 1.3 Hz, 6 H); LCMS calculated for $C_{25}H_{33}F_2N_3O_6P$ $(M+H)^+$: m/z = 540.

Step 5: [[4-((2S)-2-[(2S)-2-(acetylamino)-3-phenylpropanoyl]amino-3-amino-3-oxopropyl)phenyl](difluoro)methyl]phosphonic acid. A solution of diethyl [[4-((2*S*)-2-[(2*S*)-2-(acetylamino)-3-phenylpropanoyl]amino-3-amino-3-oxopropyl)phenyl](difluoro)methyl]phosphonate (63 mg, 0.12 mmol) in dichloromethane (1.0 mL) was treated with iodotrimethylsilane (66 μL, 0.47 mmol) and stirred for 2 h. The reaction mixture was concentrated *in vacuo* and the crude residue was purified by preparative LCMS to afford **13** (44 mg, 79%). ^{1}H NMR (500 MHz, DMSO-d_6 at 60 ^{o}C): δ 7.93 (d, *J* = 8.3 Hz, 1 H), 7.86 (d, *J* = 8.2 Hz, 1 H), 7.43 (d, *J* = 8.0 Hz, 2 H), 7.30 (d, *J*

= 8.0 Hz, 2 H), 7.23 (m, 2 H), 7.19 (m, 2 H), 7.15 (m, 1 H), (m, 2 H), 4.46 (m, 2 H), 4.46 (m, 2 H), 3.09 (dd, *J* = 14.2, 4.7 Hz, 1 H), 2.88 (dd, *J* = 14.3, 8.6 Hz, 1 H), 2.96 (dd, *J* = 14.2 Hz, 1 H), 2.72 (dd, *J* = 14.0 Hz, 1 H), 1.74 (s, 3 H). ^{13}C NMR (500 MHz, DMSO-d_6 at 60 ^{o}C): δ 172.0, 170.7, 169.2, 139.7, 137.6, 131.7, 128.7, 128.6, 127.6, 125.9, 125.5, 53.6, 53.1, 36.8, 36.7, 21.8; HRMS calculated for $C_{21}H_{25}F_2N_3O_6P$ $(M+H)^+$: m/z = 484.1453.

6- HPLC Purity Analysis by Methods A and B:

HPLC purity was determined to be >95% for all final products by the following two HPCL conditions (See Table below); 1) HPLC method A utilized a Phenominex Luna C18 column (6 x 75 mm, 3 μM particle size), with a gradient of 95% water/0.05% TFA to 5% acetonitrile/0.05% TFA at a flow rate of 1.5 mL/min over a total run time of 7 min. with UV monitoring at 220 nm and 254 nm. 2) HPLC method B utilized a Zorbax Eclipse XDB-C8 column (6 x 50 mm, 3.5 μM particle size), with a gradient of 95% water/0.05% TFA to 5% acetonitrile/0.05% TFA at a flow rate of 1.5 mL/min over a total run time of 5 min. with UV monitoring at 225 nm and 254 nm.

Compound	Formula	HPLC Analysis Data
8	$C_{23}H_{24}N_4O_6S$	Method A: t_R = 2.70 min (98.3%) Method B: t_R = 1.94 min (97.8%)
11	$C_{23}H_{26}N_4O_6S$	Method A: t_R = 2.64 min (95.5%) Method B: t_R = 1.98 min (99.0%)
12	$C_{23}H_{26}N_4O_6S$	Method A: t_R = 2.68 min (96.6%) Method B: t_R = 1.98 min (98.8%)
13	$C_{21}H_{24}F_2N_3O_6P$	Method A: t_R = 2.49 min (95.7%) Method B: t_R = 1.72 min (98.7%)