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A two stage click-based library of protein tyrosine phosphatase inhibitors

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Abstract—Protein tyrosine phosphatases (PTPs) are important regulators of signal transduction pathways. Potent and selective PTP inhibitors are useful for probing these pathways and also may serve as drugs for the treatment of a variety of diseases including type 2 diabetes and infection by the bacterium *Yersinia pestis*. In this report Cu(I)-catalyzed 'click' cycloaddition reactions between azides and alkynes were employed to generate two sequential libraries of PTP inhibitors. In the first round library methyl 4-azidobenzoylformate was reacted with 56 mono- and diynes. After hydrolysis of the methyl esters, the resulting α-ketocarboxylic acids were assayed in crude form against the *Yersinia* PTP and PTP1B. Four compounds were selected for further evaluation, and one compound was chosen as the lead for generation of the second round library. This lead compound was modified by conversion of an alcohol into an azide group, and the resulting azide was reacted with the same 56 mono- and diynes that were used in the first generation library. After screening the crude inhibitors against the *Yersinia* PTP and PTP1B, four compounds were selected and evaluated in pure form against the *Yersinia* PTP, PTP1B, TCPTP, LAR, and CD45. The best bis(α-ketocarboxylic acid) inhibitor **34** had an IC_{50} value of 550 nM against the *Yersinia* PTP and an IC_{50} value of 710 nM against TCPTP. The most potent inhibitor containing a single α-ketocarboxylic acid group **32** had IC_{50} values of 2.1, 5.7, and 2.6 μM against the *Yersinia* PTP, PTP1B, and TCPTP, respectively.

1. Introduction

1.1. PTP inhibitors

Protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs) control intracellular signal transduction pathways by regulating the phosphorylation state of tyrosine residues in proteins.[1] While the structures and biological functions of PTKs have been studied in detail, until recently PTPs have received less scrutiny. PTPs are characterized by a conserved active site sequence (H/V)**C**$(X)_5$**R**(S/T) called the PTP signature motif.[2] Based on this unique sequence, a recent survey of the human genome revealed the existence of 107 PTP genes and 81 active protein tyrosine phosphatases.[3]

Since PTPs regulate signal transduction, defective or inappropriate PTP activities play an important role in a variety of diseases including type II diabetes, cancer, dysfunctions of the immune system, and infection by pathogenic bacteria. For example, PTP1B, the first identified PTP,[4] is a negative regulator of insulin-induced glucose metabolism.[5] This PTP dephosphorylates the insulin receptor and thus shuts down the insulin signaling cascade. CD45 and TCPTP are two phosphatases that are involved in cytokine signaling and they play important roles in the immune response.[6–9] Finally, a variety of pathogenic bacteria including *Yersinia pestis* and *Salmonella typhimurium* utilize highly active PTPs as part of their virulence mechanisms. The *Y. pestis* bacterium injects its phosphatase into host cells using a type III secretion system, where it targets several focal adhesion proteins.

PTPs are attractive targets for drug development since 4% of the 'druggable genome' is thought to be phosphatases.[10] Potent and selective PTP inhibitors should be useful for probing signal transduction pathways and also as drugs for the treatment of PTP-related diseases. As a result, there is increasing effort to develop PTP inhibitors, especially since a PTP1B knockout mouse validated this enzyme as a target for the treatment of

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type II diabetes and perhaps obesity.[11,12] A number of non-hydrolyzable phosphate mimics have been developed as PTP inhibitors including aryl α-ketocarboxylic acids,[13–17] 2-(oxalylamino)benzoic acids,[18] difluoromethylenesulfonates,[19,20] squaric acids,[21] difluoromethyl enephosphonates,[22] and *O*-malonyltyrosine.[23] However, since all PTPs share the same signature motif in the catalytic domain, it is usually necessary to extend inhibitors outside of the catalytic site to gain selectivity and improve potency. Incorporating pTyr mimics into peptide templates is one method to improve the activity of inhibitors.[15,19,22,24]

PTP1B contains a second non-catalytic pTyr binding site that is formed mainly by Arg24, Arg254, Gly259, Gln262, and Met258.[25] These residues, except for Arg254 and Gln262, are not as conserved in many PTPs compared to the residues that make up the active site. This secondary site can be exploited to design inhibitors that simultaneously target both the active site and the secondary binding site. Such inhibitors often show greatly improved potency and selectivity.[26,27] Other peripheral residues, such as Arg47, Asp48, Lys41, Phe52, and Ala27, also can be targeted by extended inhibitors.[14,16,17,28–31] Our research group has recently developed two libraries of extended bidentate PTP inhibitors that are derived from α-ketocarboxylic acids.[16,17] These libraries, which were designed to target the active site and also peripheral residues, led to the identification of several potent and selective inhibitors.

1.2. Combinatorial libraries using click chemistry

The Cu(I)-catalyzed [3+2] azide–alkyne cycloaddition reaction, which Sharpless has developed into the most successful example of click chemistry, offers an expedient method to connect two components together with high yield and purity.[32–35] This reaction has become a powerful method for generating combinatorial libraries, and it has found increasing applications in bioconjugation,[36–38] lead discovery, and lead optimization.[39–41] Wong used this reaction to prepare a library of 85 human α-1,3-fucosyltransferase inhibitors, and they identified a nanomolar inhibitor that displays excellent selectivity.[39] Sharpless and coworkers used the active site of acetylcholine esterase to bind 49 azide/alkyne pairs and to catalyze the cycloaddition reaction in the absence of Cu(I).[40] Interestingly, only one pair was aligned correctly in the active site to allow the cycloaddition reaction to occur. For this pair, the enzyme served as a reaction vessel that catalyzed the formation of an extremely potent inhibitor with a dissociation constant of 77 fM.

Yao recently synthesized and screened a click-based library of PTP inhibitors that yielded a compound with an IC_{50} value of 4.7 μM against PTP1B.[42] Our current studies are designed to investigate binding interactions between inhibitors and residues outside of the PTP catalytic site. To accomplish this goal, we have constructed a library of α-ketoacid-based inhibitors using a two-stage approach. Figure 1 shows the general structure of the inhibitors. In the first generation library methyl 4-azidobenzoylformate was reacted with 56 mono- and diynes in the presence of Cu(I). Hydrolysis of the methyl esters gave α-ketoacid inhibitors, which were screened in crude form against the *Yersinia* PTP and PTP1B. We selected a lead inhibitor and modified its structure to incorporate an azide. This compound served as the starting point for the second generation library, which was prepared by reacting it with the 56 mono- and diynes. This two-stage approach yielded several inhibitors with IC_{50} values in the low micromolar range against the *Yersinia* PTP and PTP1B.

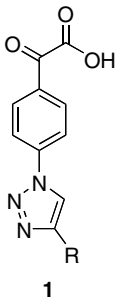

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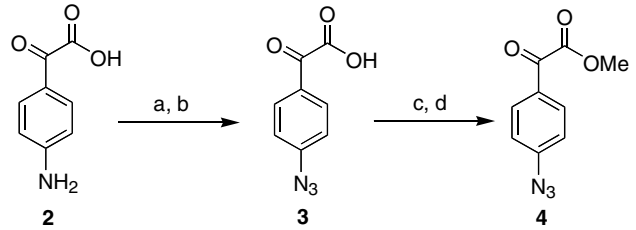
Figure 1. General structure of the inhibitors.

2. Chemistry

The first generation library required a molecule such as compound **4** (Scheme 1) that incorporated an azide group to participate in the click reaction, and an α-ketocarboxylic acid that functions as a phosphate mimic and is designed to bind in the active site of PTPs. Compound **2** was prepared from 4′-acetamidoacetophenone using the procedure of Domagala and Haskell.[43] Reaction of the aromatic amine with sodium nitrite and trifluoroacetic acid (TFA) gave the corresponding aryl diazonium salt, which was further converted to aryl azide **3** with sodium azide.[44] Azide **3** does not react with alkynes such as propiolic acid under a variety of Cu(I)-catalyzed reaction conditions.[32,45–48] This low reactivity may be caused by complexation of Cu(I) with the α-ketoacid (Fig. 2). Similar complexes have been documented in the literature.[49–54] To avoid this problem we esterified the α-ketoacid to give compound **4**.

The first generation library was synthesized as outlined in Scheme 2. Azide **4** was reacted in a 1:1 ratio with 50 alkynes and in a 2:1 ratio with six diynes (Fig. 3) to



2 3 4

Scheme 1. Reagents and conditions: (a) $NaNO_2$, TFA, 0 °C; (b) NaN_3, Et_2O; (c) $SOCl_2$, C_6H_6, reflux; (d) MeOH.

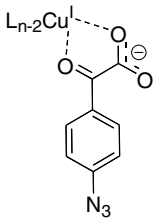
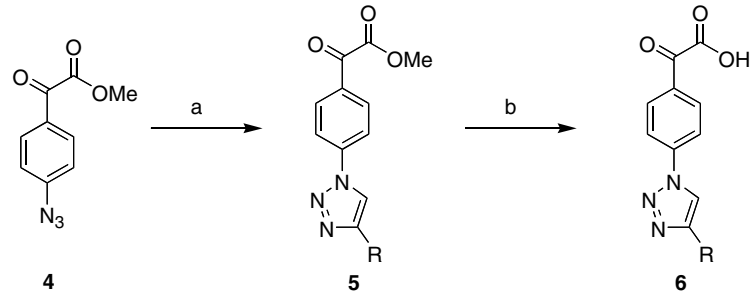


Figure 2. Possible complex between α-ketoacid **3** and Cu(I).

give triazoles **5**. Cu(I) was generated in situ using a combination of $CuSO_4$ and sodium ascorbate in the presence of the ligand tris(benzyltriazolylmethyl)amine (TBTA).[32] Alkynes **A1**–**A54** were obtained from commercial sources, while alkynes **A55** and **A56** were prepared by coupling of aminoalcohols **8** and **9** with propiolic acid (Scheme 3).[55] Aminoalcohol **8** was obtained by reduction of racemic amino acid **7**.[56] After the cycloaddition reactions were complete, the methyl



Scheme 2. Reagents: (a) alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, EtOH, *t*-BuOH, H_2O, TBTA; (b) 1 N NaOH, then 1 N HCl.

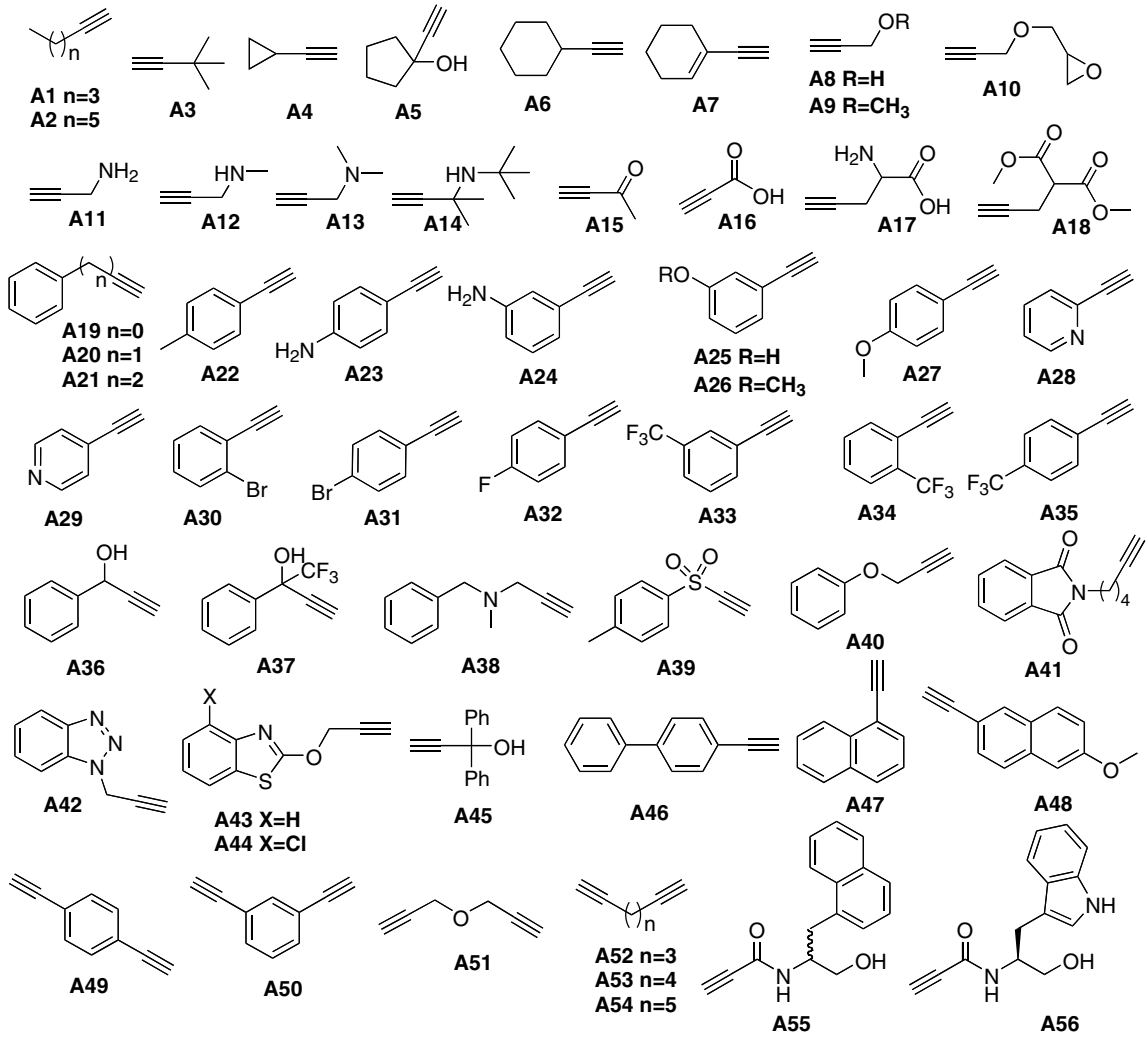
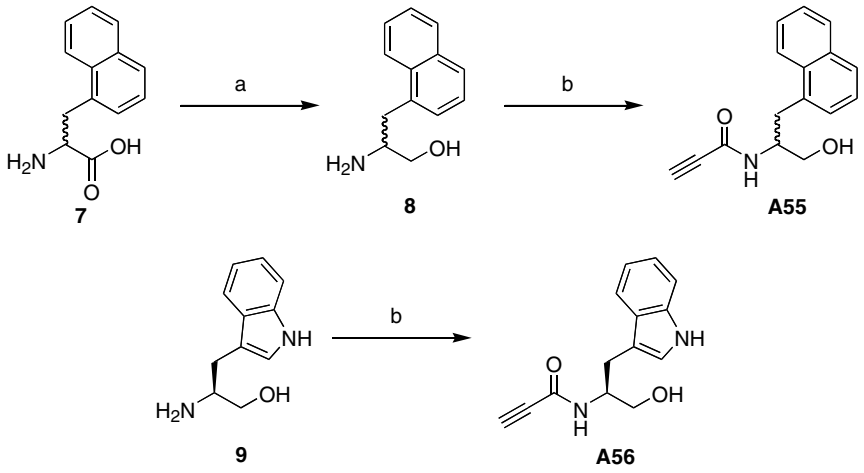


Figure 3. Alkynes used to synthesize the library.



Scheme 3. Reagents and condition: (a) $LiAlH_4$, THF, reflux; (b) propiolic acid, DCC, CH_2Cl_2.

ester groups were saponified followed by neutralization of the reaction mixtures to give the crude inhibitors **6**. The reactions were then diluted with DMSO to give a 10 mM stock solution of inhibitor based on the concentrations of starting materials used in the cycloaddition reactions, and with the assumption that the reactions proceeded to completion.

The crude inhibitors were screened at 100 μM against the *Yersinia* PTP and PTP1B. Four compounds (**11**, **13**, **15**, and **17**, corresponding to alkynes **A16**, **A46**, **A50**, and **A56**) (Fig. 4) were selected for resynthesis on a larger scale and complete characterization. The pure inhibitors were assayed against the two PTPs to obtain IC_{50} values.

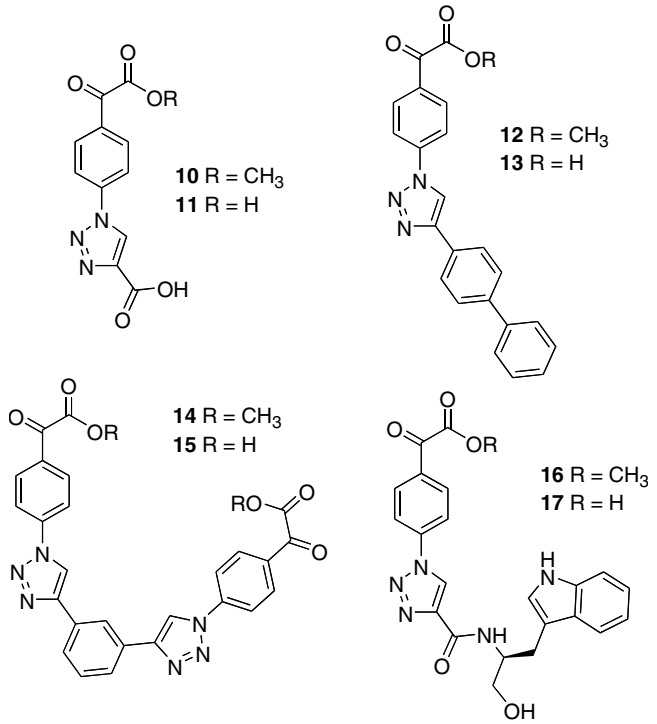
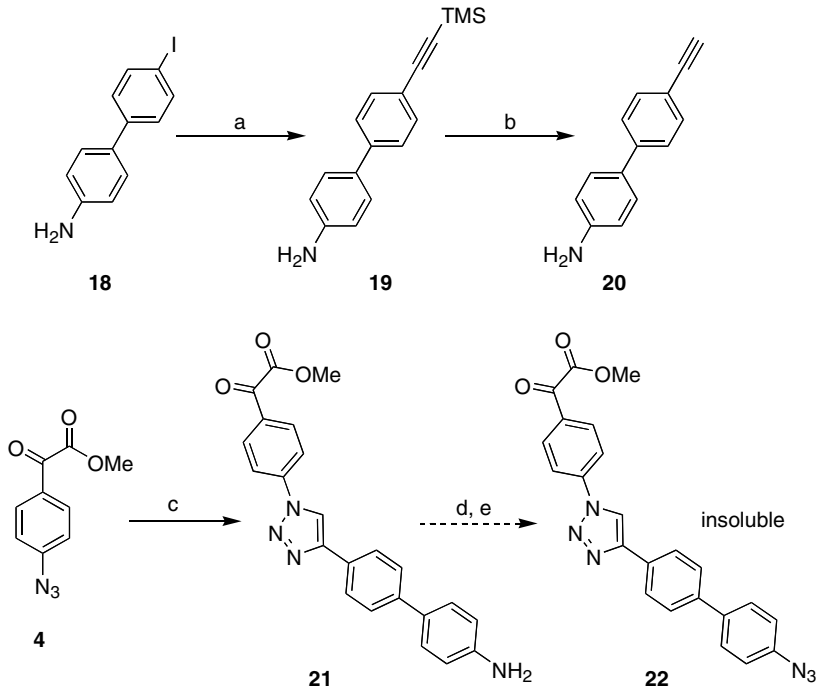


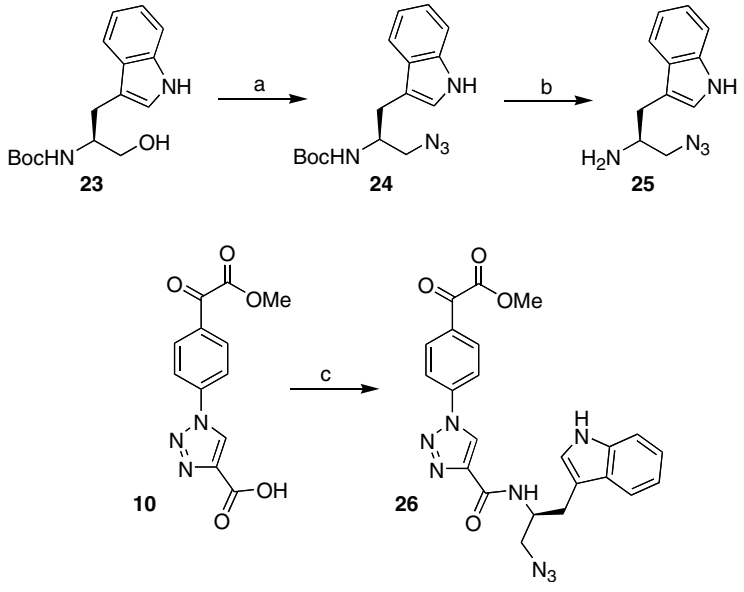
Figure 4. Four inhibitors selected for further evaluation, and their methyl ester precursors.

The enzyme assays demonstrated that, from the first generation library, compound **13** emerged as the most potent mono-α-ketoacid inhibitor. As a result, we chose this compound as the initial lead for development of the second generation library. For this library, we needed to prepare compound **22** (Scheme 4), which incorporates all of the structural elements of inhibitor **13**, but also has an additional azide group attached to the 4′-position of the biphenyl unit. The synthesis began with compound **18**, which was prepared using the procedure of Makarov and co-workers.[57] Sonogashira coupling converted the aryl iodide into the TMS-protected alkyne **19**, which was deprotected to give alkyne **20**.[58] The Cu(I)-catalyzed cycloaddition reaction between azide **4** and alkyne **20** proceeded smoothly to give triazole **21**. However, sodium nitrite oxidation of the amino group in **21**, followed by substitution of the resulting diazonium salt with sodium azide, gave a product, possibly compound **22**, which was insoluble in all standard organic solvents including DMSO. Since this compound was insoluble, it could not be used as a viable building block for the second generation library.

The inhibition studies indicated that compound **17** (Fig. 4) had similar activity against the *Yersinia* PTP as compound **13**, although it was less active against PTP1B. This observation prompted us to choose **17** as an alternate lead compound for synthesis of the second generation library. We were unable to convert compound **16** directly to azide **26** (Scheme 5) using a variety of reaction conditions.[59–67] Instead, we opted to incorporate the azide early on in the synthesis. Boc-tryptophanol **23** was converted to the corresponding azide **24** using a Mitsunobu reaction.[68] After removal of the Boc group, amine **25** was coupled with compound **10** using *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and 4-dimethylaminopyridine (DMAP) to give compound **26**.



Scheme 4. Reagents and conditions: (a) ethynyltrimethylsilane, $Pd(PPh_3)_2Cl_2$, CuI, TEA; (b) 10% NaOH, MeOH; (c) **20**, $CuSO_4{\cdot}5H_2O$, sodium ascorbate, EtOH, *t*-BuOH, H_2O, TBTA; (d) $NaNO_2$, TFA, 0 °C; (e) NaN_3, diethyl ether.

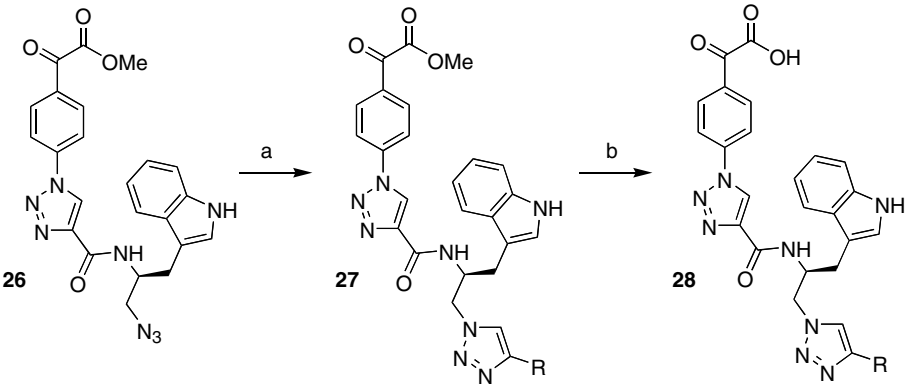


Scheme 5. Reagents: (a) PPh_3, diisopropyl azodicarboxylate (DIAD), ZnN_6, toluene; (b) TFA, CH_2Cl_2; (c) **25**, EDC, DMAP, CH_2Cl_2.

For the second generation library, azide **26** was reacted with the 56 mono- and diynes shown in Figure 3 using conditions that were similar to those used for the first generation library (Scheme 6). After saponification of the methyl esters, the crude inhibitors **28** were screened against the *Yersinia* PTP and PTP1B. Compounds **30**, **32**, **34**, and **36** (Fig. 5), corresponding to alkynes **A39**, **A48**, **A49**, and **A50**, were resynthesized on a larger scale, fully characterized, and assayed in pure form against the *Yersinia* PTP, PTP1B, TCPTP, CD45, and LAR.

3. PTP inhibition studies

The crude inhibitors from the first generation library were screened at 100 μM against the *Yersinia* PTP and PTP1B (Fig. 6). Assay mixtures contained *p*-nitrophenylphosphate (*p*-NPP) as the substrate, 50 mM 3,3-dimethylglutarate buffer at pH 7.0, 1 mM EDTA, 50 mM NaCl, and 10% DMSO. Compound **3** was included as a control and it showed 26% and 0% inhibition of the *Yersinia* PTP and PTP1B, respectively. We performed



Scheme 6. Reagents: (a) alkyne, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, acetone, *t*-BuOH, H_2O, TBTA; (b) 1 N NaOH, then 1 N HCl.

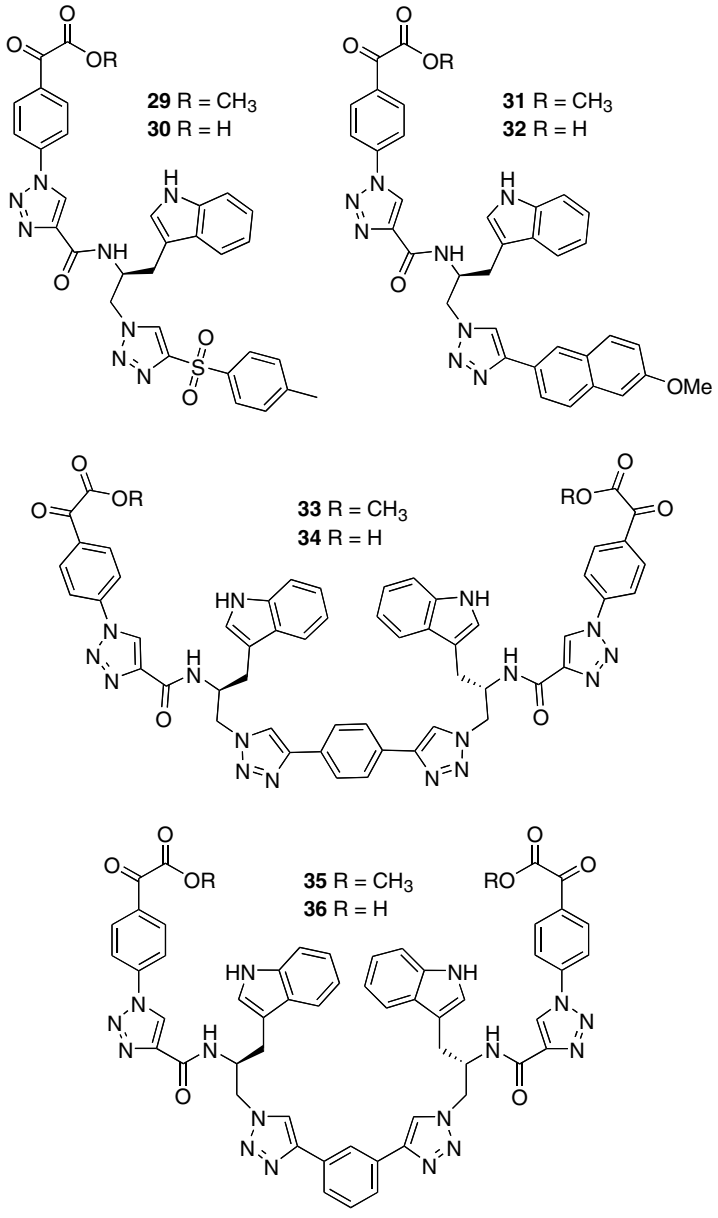


Figure 5. Four inhibitors selected for further evaluation, and their methyl ester precursors.

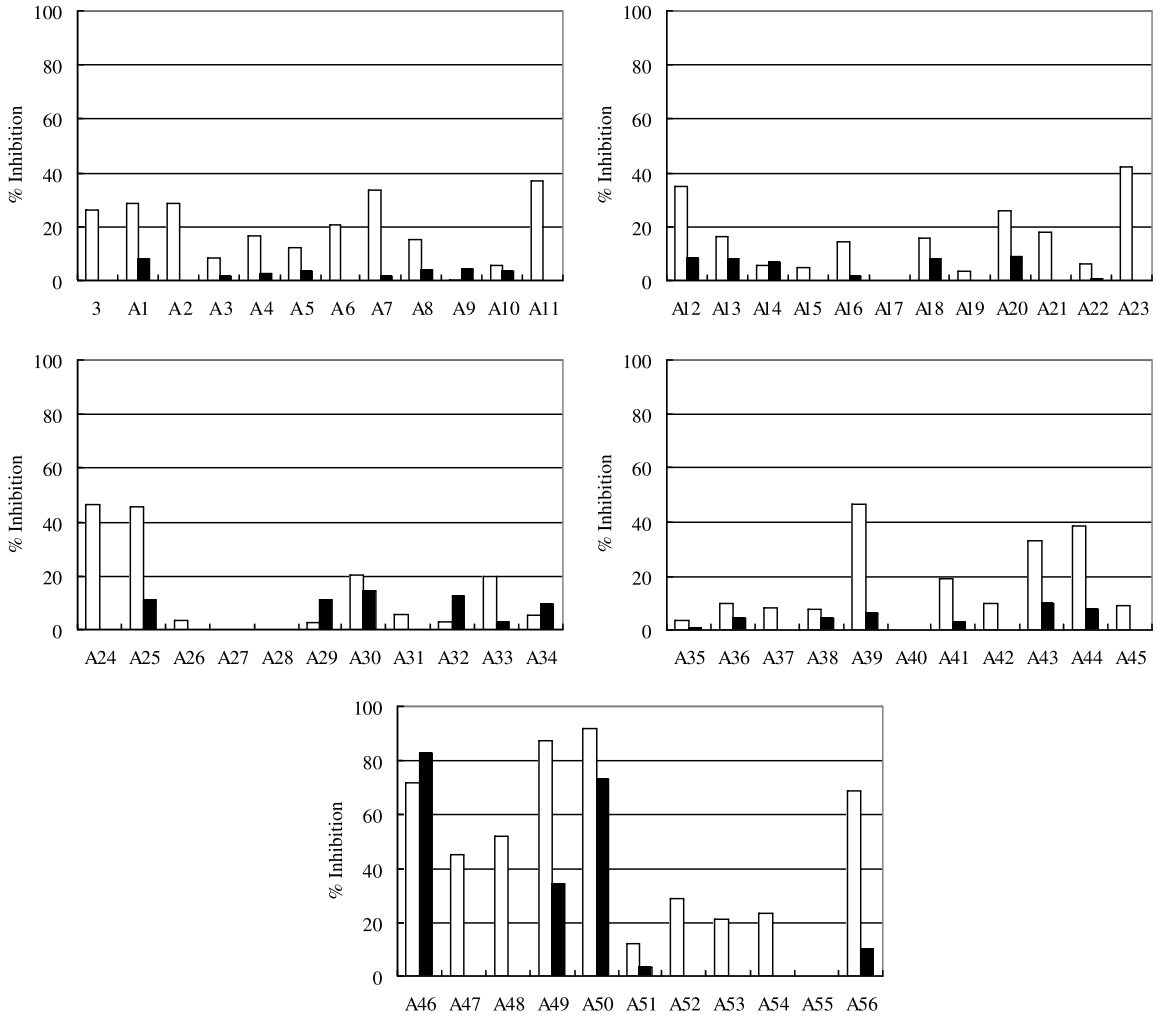


Figure 6. Assays of the first round library members at 100 μM against the *Yersinia* PTP (open bars) and PTP1B (solid bars). Designators on the *x*-axis refer to the library members generated from the alkynes shown in Figure 3.

a series of other controls for the enzyme assays that demonstrated that none of starting materials, reagents or solvents used during preparation of the library were significant inhibitors of the PTPs. One potential complication in the assay of the crude inhibitors is that α-ketoacid **3**, which is a modest PTP inhibitor, could be formed during the library synthesis by saponification of compound **4** if compound **4** was not completely consumed by reaction with the alkyne. However, TLC analyses of the cycloaddition reactions showed complete consumption of azide **4**. Thus, the concentration of α-ketoacid **3**, if it was present in the crude assay solutions, was low.

Several groups of inhibitors showed activity in the range of 40% inhibition or better. For example, small aliphatic amines such as **A11** and **A12**, and aromatic amines including **A23** and **A24** had reasonable activity. The inhibitor derived from **A25**, which is the phenol analog of aniline **A24**, was also active. The aromatic sulfoxide **A39** and the 2-alkoxybenzothiazoles **A43** and **A44** inhibit the *Yersinia* PTP, but like the other compounds from **A1** to **A45**, they are not significant inhibitors of PTP1B. Inhibitors with large aromatic surface areas such as **A46**–**A48** and **A56** showed good activity against the *Yersinia* PTP, and unlike most of the other compounds, **A46** was also active against PTP1B. Comparison of **A55**, which does not inhibit either enzyme, with **A56** suggests that the indole N–H group in **A56** may be an important contributor to binding of this compound.

Bidentate PTP inhibitors that incorporate more than one non-hydrolyzable phosphotyrosine mimic often show increased potency when compared to their monodentate analogs. This observation prompted us to include the diynes **A49**–**A54** in the library. Bidentate inhibitors derived from flexible diynes such as **A51**–**A54** were not very active. They likely encounter too large a conformational entropic penalty for binding, which offsets any advantage they gain from having two α-ketoacid groups. By contrast, the diynes **A49** and **A50** that incorporate rigid aromatic spacers have a lower entropic penalty to binding and thus have good activity against both PTPs.

Based upon the screening data we selected inhibitors derived from alkynes **A46**, **A49**, **A50**, and **A56**, which displayed greater than 60% inhibition of one or both PTPs,

for resynthesis and evaluation in purified form. However, the inhibitor generated from **A49** was insoluble in aqueous DMSO, and thus assay data are not available for this compound. Compound **3** and compound **11** (derived from **A16**) were included in the assays as controls.

The IC_{50} values for the purified inhibitors are fully consistent with the data obtained from screening of the crude inhibitors (Table 1). As expected, compounds **3** and **11** are modest inhibitors of the *Yersinia* PTP with IC_{50} values of 220 and 160 μM, respectively. In contrast, compounds **13** and **15** are good inhibitors of both PTPs. As predicted from the screening studies, compound **17** has good activity against the *Yersinia* enzyme, but is a modest inhibitor of PTP1B.

Table 1. Inhibition of phosphatases by compounds **3**, **11**, **13**, **15**, and **17**[a]

Inhibitor (alkyne)[b]	IC_{50} (μM)	
	Yersinia PTP	PTP1B
3	220 ± 20	
11 (A16)	160 ± 20	
13 (A46)	45 ± 7	84 ± 5
15 (A50)	25 ± 3	77 ± 4
17 (A56)	58 ± 4	500 ± 15

[a] Assays were performed in 50 mM 3,3-dimethylglutarate buffer at pH 7.0, 1 mM EDTA, 50 mM NaCl, and 10% DMSO.
[b] Number in brackets refers to the alkyne used to generate the inhibitor.

We initially selected inhibitor **13** as the basis for the second generation library. However as noted above, the solubility characteristics of the requisite azide-containing analog of **13**, compound **22**, made it an unsuitable building block for the library. Inhibitor **17** provided an alternate lead compound that ultimately proved successful. We did not base the second generation library on the bidentate inhibitor **15** because we wanted to obtain inhibitors with a low charge state at physiological pH to improve their chances of cell permeability. Compounds such as **15** with two α-ketoacid groups will have a double negative charge at neutral pH and are less likely to diffuse through the cell membrane than related compounds that bear only a single negative charge.

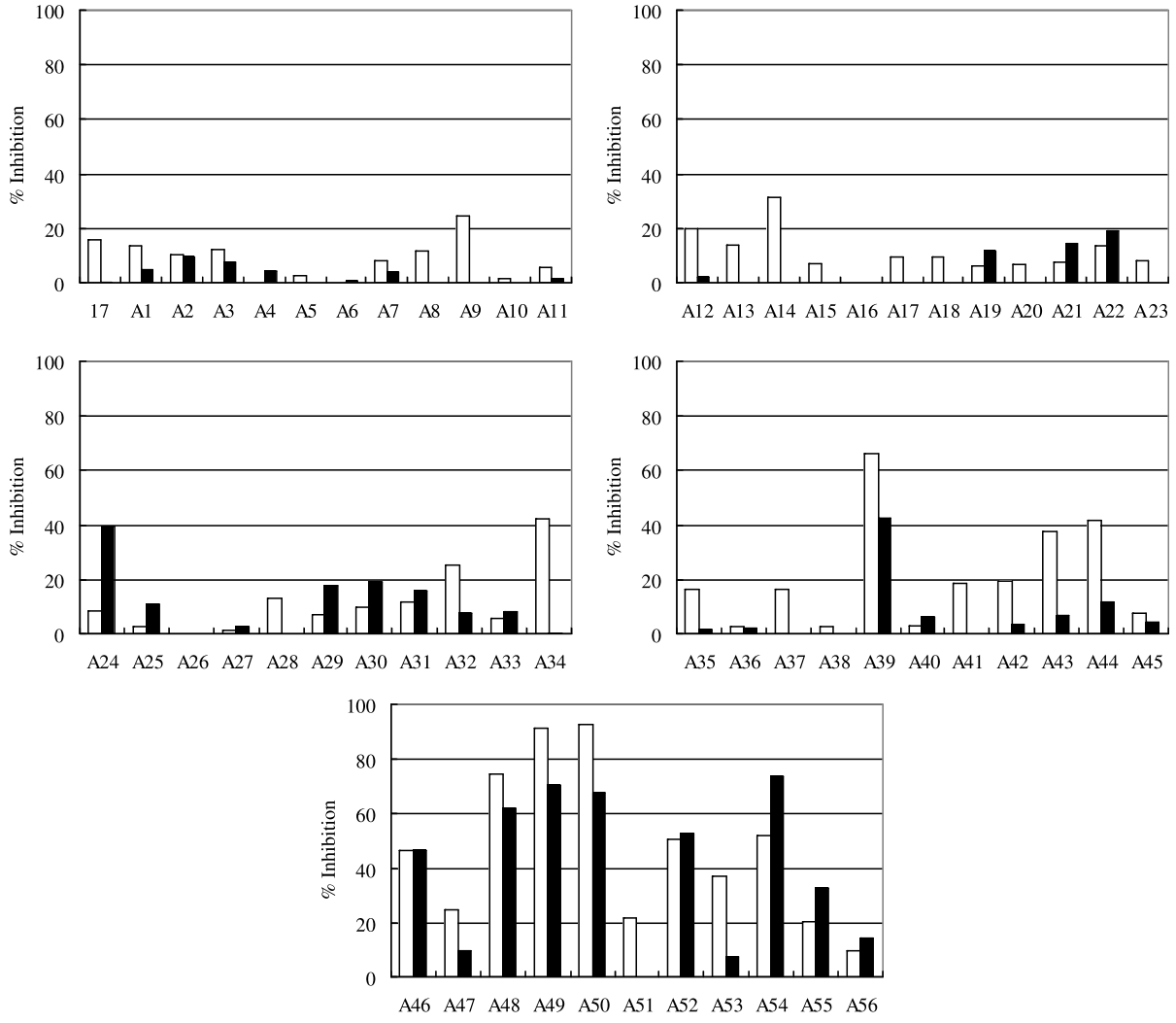


Figure 7. Assays of the second round library members at 10 μM against the *Yersinia* PTP (open bars) and PTP1B (solid bars). Designators on the *x*-axis refer to the library members generated from the alkynes shown in Figure 3.

The second generation library, which was based on inhibitor **17**, was screened against the *Yersinia* PTP and PTP1B at a concentration of 10 μM (Fig. 7). Compound **17** was used as a control. The hits from this second library with activities in the range of 40% inhibition or better are significantly more potent than inhibitors identified from the first round of screening since the second library was assayed at a 10-fold lower concentration compared to the first. It is interesting to note that many of the alkynes that yielded active inhibitors in the first generation library also gave active inhibitors in the second library. This result is somewhat surprising since the functional groups associated with each alkyne occupy different regions of the active site in the two libraries. Examples include inhibitors derived from aniline **A24**, aromatic sulfoxide **A39**, benzothiazoles **A43** and **A44**, biphenyl **A46**, and naphthalene **A48**. One plausible explanation for this observation is that these particular compounds incorporate structural features that are well suited to form strong hydrogen bonding or hydrophobic interactions with residues near the active site of the PTPs.

The only monoalkyne that yielded a hit in the second library, but not the first, is *o*-trifluoromethylphenylacetylene **A34**. Similar to the first library, the rigid diynes **A49** and **A50** yielded active bidentate inhibitors. However, unlike the first library, three of the flexible diynes (**A52**–**A54**) also gave inhibitors with significant activity. These compounds, which incorporate two α-ketoacids, may be able to bind simultaneously to the catalytic site and to the secondary pTyr binding site or to other nearby basic residues. This binding mode, with two sets of salt-bridging interactions between enzyme and inhibitor, would lead to improved activity.

We selected compounds **30**, **32**, **34**, and **36** (Fig. 5), corresponding to alkynes **A39**, **A48**, **A49**, and **A50**, respectively, for resynthesis and further evaluation. In crude form, these compounds all gave greater than 60% inhibition of the *Yersinia* PTP at 10 μM concentration. After resynthesis and characterization, we measured IC_{50} values for these inhibitors against five PTPs; the *Yersinia* PTP, PTP1B, TCPTP, CD45, and LAR (Table 2).

The inhibition profiles of the two mono-α-ketoacids (**30** and **32**) are remarkably similar. Both compounds give IC_{50} values in the range of 2–3 μM against the *Yersinia* PTP and TCPTP, 6–8 μM against PTP1B, 15–17 μM against CD45, and no inhibition of LAR at up to 100 μM. Inhibitors often have similar potencies against PTP1B and TCPTP because the active sites of these two enzymes have high homology.[69] The residues that constitute the secondary binding site in PTP1B, such as Arg24, have no counterparts in LAR and CD45. Moreover, LAR has no residue corresponding to Arg47 in PTP1B, which plays an important role for inhibitor binding in a number of studies. Thus, it is reasonable that inhibitors **30** and **32** have the lowest activities against LAR.

The most active inhibitors that we identified in this study are the bis-α-ketoacids **34** and **36**. In particular compound **34**, with a central 1,4-disubstituted benzene ring, is a potent PTP inhibitor. It has an IC_{50} of 550 nM against the *Yersinia* PTP and an IC_{50} of 710 nM against TCPTP. It is approximately 2-fold more active against the *Yersinia* PTP, PTP1B, and TCPTP when compared with inhibitor **36** that incorporates a 1,3-disubstituted benzene ring at its center. Compounds **34** and **36** have similar activities against CD45, and like the mono-α-ketoacid inhibitors, neither inhibits LAR. We performed a Lineweaver–Burk analysis of com-

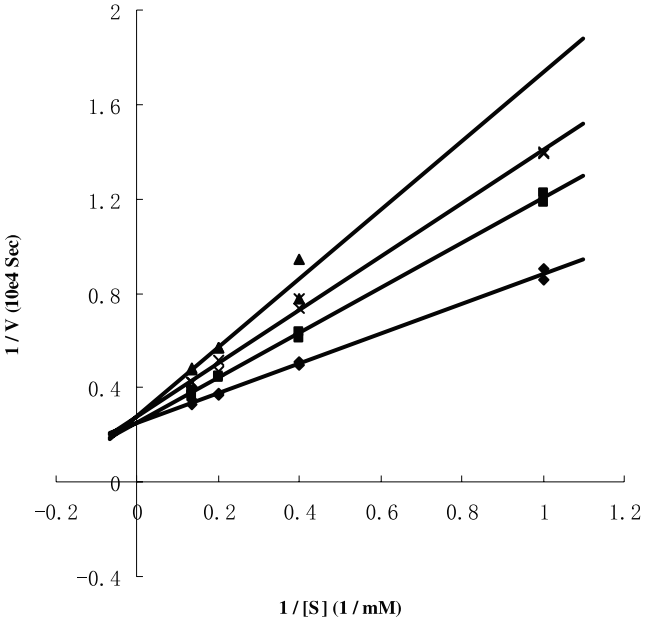


Figure 8. Inhibition of TCPTP by compound **34**. The activity of TCPTP was measured at pH 7.0 as described in Section 5 in the presence of the following concentrations of compound **34**: (♦) 0 μM; (■) 1 μM; (×) 1.5 μM; (▲) 2 μM. Substrate concentrations used in the assays were 1.0, 2.5, 5.0, and 7.5 mM.

Table 2. Inhibition of phosphatases by compounds **30**, **32**, **34**, and **36**[a]

Inhibitor (alkyne)[b]	IC_{50} (μM)				
	Yersinia PTP	PTP1B	TCPTP	CD45	LAR
30 (A39)	2.1 ± 0.8	7.4 ± 0.2	2.6 ± 0.3	15.8 ± 2	0% at 100 μM
32 (A48)	2.1 ± 0.8	5.7 ± 0.5	2.6 ± 0.3	16.8 ± 1.3	0% at 100 μM
34 (A49)	0.55 ± 0.02	2.1 ± 0.2	0.71 ± 0.03	8.4 ± 1.7	0% at 100 μM
36 (A50)	1.3 ± 0.1	4.1 ± 0.4	1.8 ± 0.1	8.5 ± 1.2	0% at 100 μM

[a] Assays were performed in 50 mM 3,3-dimethylglutarate buffer at pH 7.0, 1 mM EDTA, 50 mM NaCl, and 10% DMSO.
[b] Number in brackets refers to the alkyne used to generate the inhibitor.

pound **34** against TCPTP, which shows that the inhibitor is either a reversible competitive or perhaps mixed inhibitor of the phosphatase (Fig. 8). Fitting of the data to kinetic equations for competitive, non-competitive and uncompetitive inhibition gives a best fit to the competitive scenario.

4. Conclusions

In this report we have described the synthesis and screening of a two stage library of α-ketoacid-based PTP inhibitors. In the first stage, the Cu(I)-catalyzed cycloaddition reaction was used to append 56 different alkynes and diynes to an azide-containing pharmacophore. The best mono-ketoacid inhibitor (**13**) identified during the first round of screening showed a 5-fold improvement in activity against the *Yersinia* PTP when compared to the parent compound (**3**). The best bidentate inhibitor from the first round (**15**) was nine times more potent than compound **3**. In the second stage of library synthesis, we selected a mono-ketoacid lead compound from the first stage and modified its structure to incorporate an azide group. We performed a second round of click reactions to couple this azide with the 56 alkynes and diynes. Screening of the second stage library identified a mono-ketoacid (**32**) and a bis-ketoacid (**34**) that are 100 and 400 times more potent than the parent compound **3**, respectively.

This two stage library represents an efficient strategy to advance from a poorly active lead compound (**3**, $IC_{50} = 220\ \mu M$) to a nanomolar inhibitor of two important PTPs. It has the added advantage of requiring a relatively small number of building blocks, in this case 56 alkynes and diynes, which can be used for both stages of library synthesis. This strategy should be useful for extending inhibitors so that they take advantage of binding interactions with regions of the enzyme that are outside of the immediate region of the active site.

5. Experimental

5.1. General methods

^1H NMR and ^{13}C NMR spectra were acquired on Bruker Avance-300 or Avance-400 instruments. Chemical shifts are reported relative to TMS ($\delta = 0.00$ ppm) for ^1H NMR and $CDCl_3$ ($\delta = 77.0$ ppm) for ^{13}C NMR. Mass spectra were recorded on either a Shimadzu LCMS-QP8000 or an Applied Biosystems QSTAR electrospray mass spectrometer. Dry solvents were obtained from a Solvent Dispensing System. All reagents were used as received. Compounds **2** and **18** were prepared using procedures from the literature.[43,57] Several compounds reported herein (**14**, **21**, **31**, **33**, and **35**) are insoluble in all solvents that are commonly used to prepare samples for mass spectrometric analysis. As a result, mass spectral data have not been provided for these five compounds. Caution: All reactions involving metal azides or hydrazoic acid should be performed using the appropriate safety precautions including a fume hood and blast shield.

5.2. Compound 3

To a solution of 4.20 g (25.5 mmol) of compound **2** in 50 mL of TFA at 0 °C was added 3.50 g (50.7 mmol) of sodium nitrite in one portion. After the mixture was stirred for 2 h, 8.27 g (127 mmol) of sodium azide was added slowly over 20 min followed by the addition of 60 mL of Et_2O. The resulting mixture was stirred in the dark for an additional 2 h and the temperature was allowed to rise to room temperature. After the solvent was evaporated, the residue was dissolved in 100 mL of 1 N HCl and extracted with EtOAc (3× 100 mL). The combined organic phases were washed with brine (100 mL) and dried over $MgSO_4$. The solvent was then removed under reduced pressure. The residue was purified by chromatography using 5% acetic acid in methylene chloride as the eluent. Compound **3** was obtained as yellow solid (4.10 g, 84%). ^1H NMR (300 MHz, acetone-d_6) δ 6.35 (br s, 1H), 7.29 (d, $J = 6.8$ Hz, 2H), 8.10 (d, $J = 6.8$ Hz, 2H); ^{13}C NMR (75 MHz, acetone-d_6) δ 119.9, 129.6, 132.2, 147.1, 165.2, 186.4; HRMS-ESI ($M-H^+$) calcd for $C_8H_4N_3O_3$ 190.0253. Found: 190.0250.

5.3. Compound 4

A mixture of 3.0 g (15.7 mmol) of compound **3** and 2.3 mL (31.4 mmol) of thionyl chloride in 100 mL of anhydrous benzene was heated at reflux for 2 h. The solvent was removed under reduced pressure. The material was dissolved in 50 mL of methanol and the solution was stirred for 10 min. After the solvent was removed under reduced pressure, the residue was purified by flash chromatography (1:1 methylene chloride/hexane). Compound **4** was obtained as yellow solid (2.8 g, 87%). ^1H NMR (300 MHz, $CDCl_3$) δ 3.95 (s, 3H), 7.09 (d, $J = 8.6$ Hz, 2H), 8.01 (d, $J = 8.7$ Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 52.9, 119.3, 129.1, 132.2, 146.9, 163.7, 184.1; HRMS-ESI ($M+Na^+$) calcd for $C_9H_7N_3NaO_3$ 228.0385. Found: 228.0375.

5.4. Compound 8

To a mixture of 0.60 g (2.8 mmol) of racemic 3-(1-naphthyl)alanine in 40 mL of THF was added 0.30 g (7.9 mmol) of lithium aluminum hydride. The mixture was heated at reflux for 12 h. After the solution was cooled to room temperature, the solvent was evaporated under reduced pressure. The residue was suspended in 100 mL of EtOAc and washed with water (100 mL) and brine (100 mL). The organic phase was separated and dried over $MgSO_4$. Filtration followed by evaporation afforded compound **8** as white solid (0.50 g, 89%). The product was used in the next reaction without further purification. ^1H NMR (300 MHz, $CDCl_3$) δ 2.07 (br s, 3H), 2.93 (dd, $J = 15.0$, 9.9 Hz, 1 H), 3.29–3.37 (m, 2H), 3.51 (dd, $J = 7.8$, 6.9 Hz, 1H), 3.72 (dd, $J = 10.5$, 3.6 Hz, 1H), 7.34–7.56 (m, 4H), 7.78 (d, $J = 8.1$ Hz, 1H), 7.84–7.91 (m, 1H), 8.04–8.09 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.0, 53.3, 66.5, 123.8,

125.4, 125.7, 126.0, 127.3, 127.4, 128.9, 132.1, 134.0, 134.9; HRMS-ESI ($M+H^+$) calcd for $C_{13}H_{16}NO$ 202.1232. Found: 202.1238.

5.5. Compound A55

To a solution of 35 mg (0.498 mmol) of propiolic acid in 20 mL of methylene chloride was added 123 mg (0.598 mmol) of dicyclohexylcarbodiimide (DCC). The solution was stirred at 0 °C for 10 min. Compound **8** (100 mg, 0.498 mmol) was then added and the resulting mixture was allowed to stir for another 3.5 h. After the mixture was filtered, the solution was concentrated to dryness. The residue was purified by flash chromatography (10:1 methylene chloride/methanol) to give compound **A55** as a yellow powder (56 mg, 44%). ^1H NMR (300 MHz, $CDCl_3$) δ 2.76 (s, 1H), 2.90–3.71 (m, 5H), 4.39 (m, 1H), 6.85 (d, $J = 7.8$ Hz, 1H), 7.33–7.79 (m, 5H), 7.85 (d, $J = 7.8$ Hz, 1H), 8.27 (d, $J = 8.4$ Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 33.9, 52.5, 62.4, 73.9, 123.9, 125.4, 125.8, 126.4, 127.6, 128.8, 132.1, 133.6, 133.9, 152.6; HRMS-ESI ($M+Na^+$) calcd for $C_{16}H_{15}NNaO_2$ 276.1000. Found: 276.0992.

5.6. Compound A56

Compound **A56** was prepared from 1.0 g (5.3 mmol) of L-tryptophanol **9** by procedures analogous to the preparation of compound **A55** (yellow powder, 0.73 g, 58%, eluent: 20:1 methylene chloride/methanol). ^1H NMR (300 MHz, acetone-d_6) δ 3.02–3.20 (m, 2H), 3.49 (s, 1H), 3.72–3.74 (m, 2H), 4.35–4.47 (m, 2H), 7.05–7.24 (m, 3H), 7.44 (d, $J = 7.8$ Hz, 1H), 7.76 (d, $J = 7.8$ Hz, 1H), 7.90 (d, $J = 8.1$ Hz, 1H), 10.08 (s, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 26.4, 52.9, 62.9, 74.0, 78.1, 111.4, 111.5, 118.7, 118.8, 121.4, 123.4, 127.9, 136.8, 152.4; HRMS-ESI ($M+Na^+$) calcd for $C_{14}H_{14}N_2NaO_2$ 265.0953. Found: 265.0961.

5.7. Synthesis of the first round library

To a 1 dram vial containing a solution of 5 mg (0.024 mmol) (or 10 mg, 0.049 mmol when using diynes) of compound **4**, 0.024 mmol of alkyne or diyne, 0.4 mg (0.76 μmol) of TBTA in 0.15 ml of ethanol and 0.15 mL of *tert*-butyl alcohol was added 0.3 mg $CuSO_4{\cdot}5H_2O$ (0.0012 mmol, dissolved in 75 μL of water) followed by the addition of 1 mg of sodium ascorbate (0.0049 mmol, dissolved in 75 μL of water). The mixture was stirred for 24 h at room temperature. Once the reactions were determined to be complete by TLC analysis, 50 μL of 1 N NaOH was added. The solution was stirred for 15 min and then 50 μL of 1 N HCl was added to neutralize the reaction mixture. Based on the assumption that the yield of the reaction was 100%, DMSO (1.89 mL) was added to the vial to make a 10 mM stock solution of the inhibitor.

5.8. Representative procedure for the synthesis of compounds 10, 12, 14, and 16: compound 10

To a solution of 430 mg (2.10 mmol) of compound **4** and 147 mg (2.10 mmol) of propiolic acid dissolved in 5 mL of ethanol and 5 mL of *tert*-butyl alcohol was added 10 mg (0.0189 mmol) of TBTA followed by the addition of 53 mg of $CuSO_4{\cdot}5H_2O$ (0.212 mmol, dissolved in 2.5 mL of water) and 209 mg of sodium ascorbate (1.05 mmol, dissolved in 2.5 mL of water). The resulting mixture was allowed to stir for 24 h. Water (10 mL) was then added to the mixture and the precipitate was collected by filtration and washed with water (3× 50 mL). After the solid was dried under vacuum, compound **10** was obtained as a grey powder (500 mg, 87%). ^1H NMR (300 MHz, DMSO-d_6) δ 3.96 (s, 3H), 8.22 (m, 4H), 9.58 (s, 1H), 13.42 (br s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 53.6, 121.0, 128.0, 132.3, 132.4, 140.9, 141.5, 161.8, 163.6, 185.2; HRMS-ESI ($M+H^+$) calcd for $C_{12}H_{10}N_3O_5$ 276.0620. Found: 276.0619.

5.9. Representative procedure for the synthesis of compounds 11, 13, 15, and 17: compound 11

To a solution of 50 mg of compound **10** (0.18 mmol) in 0.5 mL of DMSO was added 0.5 mL of 1 N NaOH. The solution was allowed to stir for 15 min. After 0.5 mL of 1 N HCl was added to quench the reaction, the mixture was poured into 20 mL of ice water. The solid was collected by filtration and washed with water (2× 20 mL). After the solid was dried under vacuum, compound **11** was obtained as grey powder (36 mg, 76%). ^1H NMR (300 MHz, DMSO-d_6) δ 8.18 (d, $J = 9.0$ Hz, 2H), 8.26 (d, $J = 9.0$ Hz, 2H), 9.57 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 121.6, 128.4, 132.3, 132.9, 141.2, 141.9, 162.2, 166.3, 188.1; HRMS-ESI ($M-H^+$) calcd for $C_{11}H_6N_3O_5$ 260.0309. Found: 260.0300.

5.10. Compound 12

Compound **12** was obtained from 52 mg (0.25 mmol) of compound **4**, 45 mg (0.25 mmol) of **A46**, and 4 mg (0.0076 mmol) of TBTA by procedures analogous to the preparation of compound **10** (grey powder, 90 mg, 93%). ^1H NMR (300 MHz, DMSO-d_6) δ 3.99 (s, 3H), 7.41 (d, $J = 6.0$ Hz, 1H), 7.45–7.56 (m, 2H), 7.76 (d, $J = 6.0$ Hz, 2H), 7.85 (d, $J = 6.0$ Hz, 2H), 8.07 (d, $J = 6.0$ Hz, 2H), 8.26 (br s, 4H), 9.57 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 54.1, 120.8, 126.8, 127.5, 128.2, 128.6, 129.8, 129.9, 132.4, 133.0, 140.3, 141.0, 141.8, 148.3, 164.2, 185.7.

5.11. Compound 13

Compound **13** was obtained from 50 mg (0.13 mmol) of compound **12** by procedures analogous to the preparation of compound **11** (yellow powder, 45 mg, 93%). ^1H NMR (300 MHz, DMSO-d_6) δ 7.04 (t, $J = 6.0$ Hz, 1H), 7.48–7.53 (m, 2H), 7.76 (d, $J = 6.0$ Hz, 2H), 7.84 (d, $J = 9.0$ Hz, 2H), 8.06 (d, $J = 9.0$ Hz, 2H), 8.20 (br s, 4H), 9.54 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 120.6, 120.7, 120.8, 126.8, 127.5, 128.7, 128.6, 129.9, 132.0, 132.2, 140.3, 140.9, 141.1, 148.2, 167.3; HRMS-ESI ($M-H^+$) calcd for $C_{22}H_{14}N_3O_3$ 368.1035. Found: 368.1030.

5.12. Compound 14

Compound **14** was obtained from 33 mg (0.16 mmol) of compound **4**, 10 mg (0.079 mmol) of **A50**, and 3 mg (0.0057 mmol) of TBTA by procedures analogous to the preparation of compound **10** (grey powder, 41 mg, 95%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.98 (s, 6H), 7.68 (t, J = 7.5 Hz, 1H), 7.98 (d, J = 9.0 Hz, 2H), 8.27 (s, 8H), 8.63 (s, 1H), 9.63 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 54.0, 120.8, 121.1, 123.0, 126.4, 130.8, 131.5, 132.4, 132.9, 141.8, 148.3, 164.2, 185.7.

5.13. Compound 15

Compound **15** was obtained from 41 mg (0.064 mmol) of compound **14** by procedures analogous to the preparation of compound **11** (grey powder, 30 mg, 78%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.67 (t, J = 7.5 Hz, 1H), 7.99 (d, J = 6.0 Hz, 2H), 8.18–8.28 (m, 8H), 8.64 (s, 1H), 9.62 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 120.9, 121.1, 123.1, 126.4, 130.8, 131.6, 132.4, 141.4, 148.2, 167.3; HRMS-ESI ($M-H^+$) calcd for $C_{26}H_{15}N_6O_6$ 507.1053. Found: 507.1042.

5.14. Compound 16

Compound **16** was obtained from 190 mg (0.927 mmol) of compound **4**, 224 mg (0.926 mmol) of **A56**, and 15 mg (0.0285 mmol) of TBTA by procedures analogous to the preparation of compound **10** (yellow powder, 398 mg, 96%). ^{1}H NMR (300 MHz, acetone-d_6) δ 3.21 (d, J = 6.9 Hz, 2H), 3.77–3.80 (m, 2H), 4.02 (s, 3H), 4.23 (s, 1H), 4.50–4.51 (m, 1H), 7.02 (t, J = 7.2 Hz, 1H), 7.10 (t, J = 6.9 Hz, 1H), 7.27 (s, 1H), 7.38 (d, J = 8.1 Hz, 1H), 7.80 (d, J = 7.5 Hz, 2H), 8.24 (d, J = 8.7 Hz, 2H), 8.28 (d, J = 9.0 Hz, 2H), 9.12 (s, 1H), 10.05 (s, 1H); ^{13}C NMR (75 MHz, acetone-d_6) δ 27.1, 52.4, 53.0, 63.2, 111.7, 112.0, 119.0, 119.2, 121.0, 121.6, 123.7, 124.8, 128.4, 132.2, 132.8, 137.2, 141.5, 144.9, 159.6, 164.1, 185.3; HRMS-ESI ($M+Na^+$) calcd for $C_{23}H_{21}N_5NaO_5$ 470.1440. Found: 470.1454.

5.15. Compound 17

Compound **17** was obtained from 50 mg (0.112 mmol) of compound **16** by procedures analogous to the preparation of compound **11** (yellow powder, 40 mg, 83%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.55 (s, 1H), 2.94–3.05 (m, 2H), 3.48–3.61 (m, 2H), 4.27–4.32 (m, 1H), 6.95 (t, J = 7.5 Hz, 1H), 7.06 (t, J = 7.5 Hz, 1H), 7.16 (d, J = 2.1 Hz, 1H), 7.32 (d, J = 9.0 Hz, 1H), 7.67 (d, J = 6.0 Hz, 1H), 8.18 (d, J = 9.0 Hz, 2H), 8.24 (d, J = 9.0 Hz, 2H), 8.34 (d, J = 9.0 Hz, 1H), 9.44 (s, 1H), 10.79 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 27.3, 52.6, 63.3, 112.2, 119.0, 119.4, 121.5, 121.7, 124.0, 125.9, 128.4, 132.4, 132.8, 137.0, 141.3, 145.0, 159.7, 166.3, 188.2; HRMS-ESI ($M-H^+$) calcd for $C_{22}H_{18}N_5O_5$ 432.1308. Found: 432.1318.

5.16. Compound 19

To a slurry of 1.2 g (4.1 mmol) of compound **18**, 85 mg (0.12 mmol) of $PdCl_2(PPh_3)_2$, and 40 mg (0.21 mmol) of CuI in 20 mL of triethylamine was added 0.44 g (4.5 mmol) of ethynyltrimethylsilane. The mixture was stirred at room temperature for 1.5 h and then poured into 100 mL of saturated NH_4Cl. After extraction with EtOAc (2× 100 mL), the combined organic phases were washed with water (100 mL), brine (100 mL) and dried over $MgSO_4$. The solvent was then removed under reduced pressure. The residue was purified by flash chromatography (1:1 EtOAc/hexanes) to give compound **19** as a yellow powder (0.92 g, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.30 (s, 9H), 3.77 (s, 2H), 6.76 (d, J = 8.4 Hz, 2H), 7.44 (d, J = 8.4 Hz, 2H), 7.51 (s, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 0.1, 94.3, 105.4, 115.4, 120.8, 126.0, 127.9, 130.5, 132.4, 141.2, 146.3; HRMS-ESI (M^+) calcd for $C_{17}H_{19}NSi$ 265.1287. Found: 265.1281.

5.17. Compound 20

To a solution of 0.80 g (3.0 mmol) of compound **19** dissolved in 30 mL of methanol was added 7 mL of 10% aqueous NaOH. The solution was allowed to stir for 2 h. The organic solvent was then removed under reduced pressure and the resulting slurry was extracted with 100 mL of CH_2Cl_2. The organic phase was washed with water (100 mL), brine (100 mL) and dried over $MgSO_4$. The solvent was then evaporated to give 0.58 g (99%) of compound **20** as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.19 (s, 1H), 3.79 (s, 2H), 6.77 (d, J = 8.7 Hz, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.57 (dd, J = 13.1, 8.5 Hz, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 77.6, 84.0, 115.4, 119.8, 126.2, 128.0, 130.3, 132.6, 141.6, 146.4; HRMS-ESI (M^+) calcd for $C_{14}H_{11}N$ 193.0891. Found: 193.0886.

5.18. Compound 21

Compound **21** was obtained from 0.62 g (3.0 mmol) of compound **4**, 0.58 g (3.0 mmol) of compound **20**, 48 mg (0.091 mmol) of TBTA, 75 mg (0.30 mmol) of $CuSO_4{\cdot}5H_2O$ and 240 mg (1.21 mmol) of sodium ascorbate by procedures analogous to the preparation of compound **10** (yellow solid, 1.13 g, 94%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.98 (s, 3H), 6.66 (d, J = 5.7 Hz, 2H), 7.46 (d, J = 6.9 Hz, 2H), 7.70 (d, J = 7.2 Hz, 2H), 7.96 (d, J = 6.9 Hz, 2H), 8.24 (s, 4H), 9.48 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 54.0, 115.2, 120.2, 120.7, 126.6, 126.7, 127.9, 132.3, 132.9, 141.5, 141.8, 148.6, 164.2, 185.7.

5.19. Compound 24

To a solution of 1.08 g (3.74 mmol) of Boc-tryptophanol and 1.96 g (7.48 mmol) of PPh_3 in 40 mL of toluene was added 0.86 g (2.80 mmol) of $ZnN_6{\cdot}2Py$. DIAD (1.51 g, 7.48 mmol) was then added dropwise into the slurry. The reaction mixture was stirred for 1 h and then the solid was removed by filtration. After the toluene was removed under reduced pressure, the residue was purified by flash chromatography (2.5:1 hexanes/EtOAc) to give compound **24** as white solid (1.11 g, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.54 (s, 9H), 2.95–3.02 (m, 2H), 3.29–3.44 (m, 2H), 4.15 (br s,

1H), 4.94–5.05 (m, 1H), 7.00 (d, $J = 1.2$ Hz, 1H), 7.15–7.28 (m, 2H), 7.39 (d, $J = 7.8$ Hz, 1H), 7.69 (d, $J = 7.8$ Hz, 1H), 8.70 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.5, 50.8, 53.3, 70.2, 79.9, 110.8, 111.5, 118.8, 119.6, 122.1, 123.2, 127.6, 136.4, 155.6; HRMS-ESI ($M+Na^+$) calcd for $C_{16}H_{21}N_5NaO_2$ 338.1593. Found: 338.1585.

5.20. Compound 25

Compound **24** (1.1 g, 3.5 mmol) was stirred with 50 mL of 50% TFA in methylene chloride for 30 min. The solvent was then removed and the residue was purified by flash chromatography (20:1 methylene chloride/methanol) to give compound **25** as a brown oil (0.45 g, 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79 (br s, 2H), 2.80 (dd, $J = 14.0$, 7.6 Hz, 1H), 2.98 (dd, $J = 14.4$, 5.2 Hz, 1H), 3.26–3.34 (m, 2H), 3.47 (dd, $J = 11.2$, 3.6 Hz, 1H), 7.07 (d, $J = 1.6$ Hz, 1H), 7.17 (t, $J = 7.2$ Hz, 1H), 7.24 (t, $J = 7.2$ Hz, 1H), 7.40 (d, $J = 8.0$ Hz, 1H), 7.63 (d, $J = 8.0$ Hz, 1H), 8.37 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 30.9, 52.3, 57.6, 111.3, 111.9, 118.8, 119.6, 122.2, 122.8, 127.5, 136.4; HRMS-ESI ($M+H^+$) calcd for $C_{11}H_{14}N_5$ 216.1249. Found: 216.1257.

5.21. Compound 26

To a solution of 0.38 g (1.4 mmol) of compound **10**, 0.27 g (1.4 mmol) of EDC, and 0.17 g (1.4 mmol) of DMAP dissolved in 50 mL of methylene chloride was added 0.30 g (1.4 mmol) of compound **25**. The reaction mixture was stirred overnight at room temperature. The solvent was then removed and the residue was dissolved in 100 mL of Et_2O. The solution was washed with 100 mL of 1 N HCl and 100 mL of brine. The organic layer was dried over $MgSO_4$ and concentrated to dryness. The residue was purified by flash chromatography (100:2 methylene chloride/acetic acid) to afford compound **26** as grey powder (0.41 g, 62%). ^{1}H NMR (300 MHz, acetone-d_6) δ 2.90–3.25 (m, 2H), 3.65–3.72 (m, 2H), 4.02 (s, 3H), 4.60–4.71 (m, 1H), 7.01–7.13 (m, 2H), 7.30 (d, $J = 2.1$ Hz, 1H), 7.39 (d, $J = 8.1$ Hz, 1H), 7.74 (d, $J = 7.8$ Hz, 1H), 7.96–7.99 (d, $J = 8.4$ Hz, 1H), 8.22–8.27 (m, 4H), 9.10 (s, 1H), 10.09 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.8, 49.8, 52.5, 53.5, 110.8, 111.4, 118.5, 118.8, 120.7, 121.4, 123.4, 124.5, 127.8, 131.7, 132.5, 136.8, 141.1, 144.3, 159.2, 163.7, 184.9; HRMS-ESI ($M+Na^+$) calcd for $C_{23}H_{20}N_8NaO_4$ 495.1505. Found: 495.1502.

5.22. Synthesis of the second round library

To a 1 dram vial containing a solution of 5 mg (0.011 mmol) (or 10 mg, 0.022 mmol, when using diynes) of compound **26**, 0.011 mmol of alkyne or diyne, 0.5 mg (0.94 nmol) of TBTA in 0.2 ml of acetone and 0.1 mL of *tert*-butyl alcohol was added 0.3 mg $CuSO_4{\cdot}5H_2O$ (0.0012 mmol, dissolved in 100 μL of water) and 1 mg of sodium ascorbate (0.0049 mmol, dissolved in 100 μL of water). The mixture was stirred for 24 h at room temperature. Once the reactions were determined to be complete by TLC analysis, 100 μL of 1 N NaOH was added. The solution was stirred for 15 min and then 100 μL of 1 N HCl was added to neutralize the reaction mixture. Based on the assumption that the yield of the reaction was 100%, DMSO (1.418 mL) was added to the vial to make a 5 mM stock solution of the inhibitor.

5.23. Representative procedure for the synthesis of compounds 29, 31, 33, and 35: compound 29

To a solution of 25 mg (0.053 mmol) of compound **26** and 9.5 mg (0.053 mmol) of **A39** dissolved in 1 mL of acetone and 1 mL of *tert*-butyl alcohol was added 1 mg (0.0019 mmol) of TBTA followed by the addition of 1.3 mg $CuSO_4{\cdot}5H_2O$ (0.053 mmol, dissolved in 0.5 mL of water) and 5.3 mg of sodium ascorbate (0.027 mmol, dissolved in 0.5 mL of water). The resulting mixture was allowed to stir for 24 h. Water (10 mL) was then added to the mixture and the precipitate was collected by filtration and washed with water (2× 20 mL). After the solid was dried under vacuum, compound **29** was obtained as grey powder (30 mg, 87%). ^{1}H NMR (300 MHz, acetonitrile-d_3) δ 2.31 (s, 3H), 3.17–3.20 (m, 2H), 4.00 (s, 3H), 4.62–4.81 (m, 3H), 7.06 (t, $J = 6.9$ Hz, 1H), 7.14 (t, $J = 6.9$ Hz, 1H), 7.20 (d, $J = 2.4$ Hz, 1H), 7.32 (d, $J = 8.4$ Hz, 2H), 7.40 (d, $J = 8.1$ Hz, 1H), 7.64 (t, $J = 7.8$ Hz, 2H), 7.78 (d, $J = 8.4$ Hz, 2H), 8.02 (d, $J = 9.0$ Hz, 2H), 8.24 (d, $J = 9.0$ Hz, 2H), 8.39 (s, 1H), 8.65 (s, 1H), 9.18 (s, 1H); ^{13}C NMR (75 MHz, acetonitrile-d_3) δ 20.6, 27.4, 50.0, 52.9, 53.6, 110.1, 111.4, 118.4, 119.1, 120.7, 121.6, 123.7, 124.4, 127.4, 127.5, 128.0, 129.9, 131.8, 132.7, 136.5, 137.7, 140.8, 143.4, 145.3, 148.2, 159.2, 163.4, 184.9; HRMS-ESI ($M+Na^+$) calcd for $C_{32}H_{28}N_8NaO_6S$ 675.1750. Found: 675.1760.

5.24. Representative procedure for the synthesis of compounds 30, 32, 34, and 36: compound 30

To a solution of 30 mg of compound **29** (0.046 mmol) dissolved in 0.5 mL of DMSO was added 0.5 mL of 1 N NaOH. The solution was allowed to stir for 15 min. After 0.5 mL of 1 N HCl was added to quench the reaction, the mixture was poured into 20 mL of ice water. The solid was collected by filtration and washed with water (2× 20 mL). After the solid was dried under vacuum, compound **30** was obtained as grey powder (25 mg, 85%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.24 (s, 3H), 3.01–3.19 (m, 2H), 4.65–4.88 (m, 3H), 6.96 (t, $J = 6.9$ Hz, 1H), 7.06 (t, $J = 6.9$ Hz, 1H), 7.21 (d, $J = 1.8$ Hz, 1H), 7.31–7.34 (m, 3H), 7.61 (d, $J = 7.8$ Hz, 1H), 7.70 (d, $J = 8.4$ Hz, 2H), 8.12 (d, $J = 8.7$ Hz, 2H), 8.18 (d, $J = 8.7$ Hz, 2H), 8.89 (d, $J = 8.7$ Hz, 1H), 8.92 (s, 1H), 9.29 (s, 1H), 10.89 (s, 1H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 21.8, 28.5, 50.6, 54.1, 110.8, 112.3, 119.2, 121.3, 121.8, 124.4, 126.0, 128.0, 128.1, 128.9, 129.6, 130.8, 132.0, 133.9, 137.0, 138.2, 140.6, 144.4, 145.5, 148.0, 159.8, 167.7, 191.0; HRMS-ESI ($M+Na^+$) calcd for $C_{31}H_{26}N_8NaO_6S$ 661.1594. Found: 661.1610.

5.25. Compound 31

Compound **31** was obtained from 25 mg (0.053 mmol) of compound **26**, 9.6 mg (0.053 mmol) of **A48**, and 1 mg (0.0076 mmol) of TBTA by procedures analogous to the preparation of compound **29** (grey powder, 31 mg, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.11–3.17 (m, 2H), 3.87 (s, 3H), 3.95 (s, 3H), 4.73–4.77 (m, 3H), 6.99 (t, J = 7.6 Hz, 1H), 7.07 (t, J = 7.4 Hz, 1H), 7.16 (dd, J = 8.8, 2.0 Hz, 1H), 7.25 (d, J = 2.0 Hz, 1H), 7.31 (d, J = 2.0 Hz, 1H), 7.34 (d, J = 8.0 Hz, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.82–7.89 (m, 3H), 8.19 (s, 4H), 8.25 (s, 1H), 8.60 (s, 1H), 8.95 (d, J = 8.8 Hz, 1H), 9.36 (s, 1H), 10.89 (br s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 51.0, 54.0, 56.0, 59.2, 119.2, 120.0, 121.4, 121.9, 122.8, 124.2, 124.4, 124.9, 126.2, 126.8, 128.2 (two overlapping peaks), 129.3, 130.3, 130.8, 132.7, 132.8, 134.6, 137.0, 141.3, 144.6, 147.1, 157.3, 158.2, 159.9, 164.0, 185.6.

5.26. Compound 32

Compound **32** was obtained from 31 mg (0.047 mmol) of compound **31** by procedures analogous to the preparation of compound **30** (grey powder, 27 mg, 89%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.09–3.17 (m, 2H), 3.87 (s, 3H), 4.73–4.85 (m, 3H), 7.02 (t, J = 7.4 Hz, 1H), 7.08 (t, J = 7.4 Hz, 1H), 7.16 (dd, J = 8.8, 2.0 Hz, 1H), 7.26 (d, J = 2.0 Hz, 1H), 7.28 (d, J = 2.0 Hz, 1H), 7.34 (d, J = 7.6 Hz, 1H), 7.66 (d, J = 8.0 Hz, 1H), 7.82–7.90 (m, 4H), 8.08–8.16 (m, 3H), 8.26 (s, 1H), 8.61 (s, 1H), 8.98 (d, J = 8.8 Hz, 1H), 9.35 (s, 1H), 10.89 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.6, 51.0, 53.4, 56.0, 106.8, 111.1, 112.3, 119.2, 120.0, 121.5, 121.8, 122.7, 124.2, 124.4, 124.9, 126.2, 126.9, 128.2 (two overlapping peaks), 129.3, 130.3, 131.8, 134.6, 137.0, 140.9, 144.6, 147.1, 158.2, 159.9; HRMS-ESI ($M-H^+$) calcd for $C_{35}H_{27}N_8O_5$ 639.2104. Found: 639.2118.

5.27. Compound 33

Compound **33** was obtained from 30 mg (0.064 mmol) of compound **26**, 4 mg (0.032 mmol) of **A49** and 2 mg (0.0076 mmol) of TBTA by procedures analogous to the preparation of compound **29** (grey powder, 34 mg, 100%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.05–3.16 (m, 4H), 3.95 (s, 6H), 4.68–4.82 (m, 6H), 6.98 (t, J = 7.2 Hz, 2H), 7.07 (t, J = 7.2 Hz, 2H), 7.24 (s, 2H), 7.33 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 7.6 Hz, 2H), 7.84 (s, 4H), 8.19 (s, 8H), 8.56 (s, 2H), 8.92 (d, J = 8.4 Hz, 2H), 9.36 (s, 2H), 10.86 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.5, 51.0, 53.4, 54.0, 111.0, 112.3, 119.2, 121.4, 121.8, 122.9, 124.4, 126.3, 126.4, 128.2, 131.0, 132.7, 137.0, 141.4, 144.6, 146.6, 159.8, 164.0, 185.6.

5.28. Compound 34

Compound **34** was obtained from 34 mg (0.032 mmol) of compound **33** by procedures analogous to the preparation of compound **30** (grey powder, 28 mg, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.06–3.17 (m, 4H), 4.68–4.83 (m, 6H), 6.99 (t, J = 7.6 Hz, 2H), 7.07 (t, J = 7.6 Hz, 2H), 7.25 (s, 2H), 7.34 (d, J = 8.0 Hz, 2H), 7.65 (d, J = 7.6 Hz, 2H), 7.85 (s, 4H), 8.19 (s, 8H), 8.57 (s, 2H), 8.94 (d, J = 8.4 Hz, 2H), 9.34 (s, 2H), 10.88 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 29.0, 51.0, 53.4, 111.0, 112.3, 119.2, 121.5, 121.8, 122.9, 124.4, 126.2, 126.4, 128.2, 131.0, 132.3, 137.0, 140.0, 141.1, 144.6, 146.6, 159.8; HRMS-ESI ($M-H^+$) calcd for $C_{54}H_{41}N_{16}O_8$ 1041.3293. Found: 1041.3265.

5.29. Compound 35

Compound **35** was obtained from 30 mg (0.064 mmol) of compound **26**, 4 mg (0.032 mmol) of **A50** and 2 mg (0.0076 mmol) of TBTA by procedures analogous to the preparation of compound **29** (grey powder, 33 mg, 97%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.15–3.19 (m, 4H), 3.95 (s, 6H), 4.68–4.83 (m, 6H), 6.98 (t, J = 7.4 Hz, 2H), 7.06 (t, J = 7.2 Hz, 2H), 7.24 (s, 2H), 7.33 (d, J = 8 Hz, 2H), 7.44 (t, J = 7.6 Hz, 1H), 7.64 (d, J= 7.6 Hz, 2H), 7.70 (d, J = 7.6 Hz, 2H), 8.18 (s, 8H), 8.28 (s, 1H), 8.61 (s, 2H), 8.91 (d, J = 8.4 Hz, 2H), 9.34 (s, 2H), 10.85 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.5, 51.0, 53.5, 54.0, 111.0, 112.3, 119.2, 121.4, 121.8, 122.5, 123.1, 124.4, 125.3, 126.2, 128.2, 130.3, 132.2, 132.7, 137.0, 141.4, 144.6, 146.7, 159.8, 164.0, 185.6.

5.30. Compound 36

Compound **36** was obtained from 33 mg (0.031 mmol) of compound **35** by procedures analogous to the preparation of compound **30** (grey powder, 26 mg, 81%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.01–3.21 (m, 4H), 4.68–4.84 (m, 6H), 7.00 (t, J = 7.4 Hz, 2H), 7.06 (t, J = 7.5 Hz, 2H), 7.23 (s, 2H), 7.33 (d, J = 8.1 Hz, 2H), 7.43 (t, J = 7.6 Hz, 1H), 7.64 (d, J= 7.5 Hz, 2H), 7.70 (d, J = 7.8 Hz, 2H), 8.16 (s, 8H), 8.27 (s, 1H), 8.60 (s, 2H), 8.90 (d, J = 8.4 Hz, 2H), 9.28 (s, 2H), 10.85 (s, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 28.6, 50.9, 53.5, 111.0, 112.3, 119.2, 121.2, 121.6, 121.8, 122.5, 123.0, 124.4, 125.3, 126.2, 128.2, 130.3, 131.8, 132.2, 137.0, 141.0, 144.5, 146.6, 159.8; HRMS-ESI ($M-H^+$) calcd for $C_{54}H_{41}N_{16}O_8$ 1041.3293. Found: 1041.3260.

5.31. PTP assays

The phosphatase activities of the *Yersinia* PTP, PTP1B, LAR, TCPTP, and CD45 were assayed using *p*-nitrophenyl phosphate (*p*-NPP) as the substrate at room temperature and the reaction progress was monitored by UV spectroscopy. Initial rates were determined by monitoring the hydrolysis of *p*-NPP at 405 nm, from 10 to 130 s after mixing. Assay solutions contained 50 mM 3,3-dimethylglutarate at pH 7.0, 1 mM EDTA, 50 mM NaCl, and 10% DMSO. For percent inhibition assays, the substrate concentrations were kept at 2.9 and 2.0 mM for *Yersinia* PTP and PTP1B, respectively. For IC_{50} assays, the substrate concentrations were kept at K_m. The K_m values in this buffer were determined to be 2.9, 2.0, 2.3, 2.1, and 7.0 mM, for the *Yersinia* PTP, PTP1B, LAR, TCPTP, and CD45, respectively. IC_{50} values were calculated using a Dixon analysis. Data analysis was performed using the commercial graphing package Grafit (Erithacus Software, Ltd.). This pro-

gram was used to fit the data shown in Figure 8 to the kinetic equation for competitive inhibition, and it gave an inhibition constant of 1.41 ± 0.12 μM.

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Supplementary data

^{1}H and ^{13}C NMR spectra for all new compounds are available in the supporting information.

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