Investigation of Potential Bioisosteric Replacements for the Carboxyl Groups of Peptidomimetic Inhibitors of Protein Tyrosine Phosphatase 1B: Identification of a Tetrazole-Containing Inhibitor with Cellular Activity

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Protein tyrosine phosphatases (PTPs) constitute a diverse family of enzymes that, together with protein tyrosine kinases, control the level of intracellular tyrosine phosphorylation, thus regulating many cellular functions. PTP1B negatively regulates insulin signaling, in part, by dephosphorylating key tyrosine residues within the regulatory domain of the β-subunit of the insulin receptor, thereby attenuating receptor kinase activity. Inhibitors of PTP1B would therefore have the potential of prolonging the phosphorylated (activated) state of the insulin receptor and are anticipated to be a novel treatment of the insulin resistance characteristic of type 2 diabetes. We previously reported a series of small molecular weight peptidomimetics as competitive inhibitors of PTP1B, with the most active analogues having K_i values in the low nanomolar range. Furthermore, we confirmed that the O-carboxymethyl salicylic acid moiety is a remarkably effective novel phosphotyrosine mimetic. Because of the low cell permeability of this compound class, it was important to investigate the possibility of replacing one or both of the remaining carboxyl groups while maintaining PTP1B inhibitory activity. The analogues described herein further support the importance of an acidic functionality at both positions of the tyrosine head moiety. An important discovery was the ortho tetrazole analogue **29** (K_i = 2.0 μM), which was equipotent to the dicarboxylic acid analogue **2** (K_i = 2.0 μM). Solution of the X-ray cocrystal structure of the ortho tetrazole analogue **29** bound to PTP1B revealed that the tetrazole moiety is well-accommodated in the active site and binds in a fashion similar to the ortho carboxylate analogue **2** reported previously. This novel monocarboxylic acid analogue revealed significantly higher Caco-2 cell permeability as compared to all previous compounds. Furthermore, compound **29** exhibited modest enhancement of insulin-stimulated 2-deoxyglucose uptake by L6 myocytes.

Introduction

Insulin resistance is central to type 2 diabetes and is known to involve decreased tyrosine phosphorylation of insulin receptors (IR) despite normal insulin levels.[1,2] The biological actions of insulin are initiated when insulin binds to the α-subunit of its receptor, resulting in stimulation of intrinsic receptor tyrosine kinase activity, autophosphorylation of the β-subunit, and the subsequent phosphorylation of intracellular substrates.[3] In the insulin resistant state in type 2 diabetes, insulin binding to its receptor is usually normal but the insulin-signaling cascade is attenuated, due to a defect probably at the level of the IR itself.[4]

Protein tyrosine phosphatases (PTPs) constitute a diverse family of enzymes and are responsible for the selective dephosphorylation of tyrosine residues.[5] Several PTPs, including PTP1B, LAR, PTPα, and PTPϵ, are capable of dephosphorylating the IR, and thereby attenuating tyrosine kinase activity.[6−10] However, in one case, LAR knockout mice have not shown altered glucose homeostasis.[11] Furthermore, PTP1B has been implicated in the insulin resistance associated with diabetes and obesity by the finding of correlations between insulin resistance and the levels of PTP1B in muscle and adipose tissue.[12,13] This is further supported by a variety of cellular and biochemical studies where PTP1B has been shown to play a role in the dephosphorylation of the IR.[14] Therefore, the use of specific PTP1B inhibitors may enhance insulin action and represents a novel strategy for the treatment of type 2 diabetes. A recent study with PTP1B knockout mice supports this hypothesis by demonstrating that the loss of PTP1B activity in vivo results in an enhancement of insulin sensitivity and decreased susceptibility to diet-induced obesity.[15]

The development of specific PTP1B inhibitors to treat type 2 diabetes has been the subject of intensive research in recent years. Most progress toward specific inhibitors has been made in the development of reversible competitive inhibitors that mimic the natural substrate, a phosphotyrosine-containing polypeptide.[16,17] In general, most of these inhibitors suffer from low bioavailability, even though a few investigators have reported small molecules with in vivo activity in diabetic mice.[18] Because of the electrostatic properties of the

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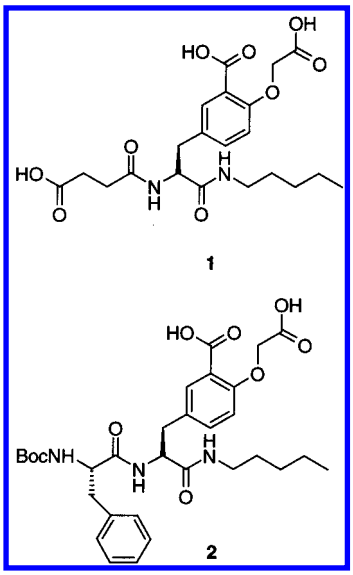
active site, it has proven difficult to develop an effective phosphotyrosine mimetic with a formal charge of less than −2 at physiological pH.

Previously, we reported the discovery that a simple tripeptide Ac-NH-Asp-Tyr(SO_3H)-Nle-NH_2 is a relatively potent competitive inhibitor of PTP1B (K_i = 5 μM).[19,20] An analogue program around this compound class was launched with the aim of attenuating the peptidic character of the lead compound. This led to a series of bisamides with the most potent compounds having K_i values in the submicromolar range.[20] An important discovery was the novel phosphotyrosine mimic, O-carboxymethyl salicylic acid,[21] seen in compound **1** (K_i = 6.5 μM). This novel phosphotyrosine bioisostere was independently reported by Burke et al. who incorporated it into hexapeptides.[22] Furthermore, structure−activity relationship investigations of the N-terminal side chain showed that the p-1 aspartic acid could be successfully replaced by phenylalanine so that compounds with neutral N termini could be developed, which maintained good inhibitory activity, e.g., compound **2** (K_i = 2.0 μM).[20]

In this study, we have focused on the tyrosine headgroup. Because cell permeability is a key issue for these compounds, it was important to investigate the possibility of replacing one or both of the remaining carboxyl groups while maintaining PTP1B inhibitory activity. This report describes the synthesis and inhibition activities of a series of analogues of **1** and **2**, wherein the carboxylic acids have been replaced with various groups with the primary aim of increasing permeability and potency.



Chemistry

By a methodology similar to that previously described,[20] analogues of **2** could be prepared as shown in Scheme 1. Carbonylative palladium-catalyzed coupling of the ortho iodo tyrosine intermediate **3**, in either benzyl alcohol or methanol, generated the corresponding esters **4** and **5**. Alkylation with methyl bromoacetate or benzyl bromoacetate afforded compounds **6** and **7**, respectively. Deprotection of the Boc group with trifluoroacetyl (TFA) and coupling with Boc-L-phenylalanine under standard carbodiimide conditions generated compounds **8** and **9**. Catalytic hydrogenolysis of the benzyl esters then afforded the pure target compounds **10** and **11**. The compounds **10** and **11** were activated with 1,1′-carbonyldiimidazole (CDI) in refluxing tetrahydrofuran (THF) and reacted with hydroxylamine,[23] which generated the corresponding hydroxamates **12** and **13**, respectively. Hydrolysis with aqueous LiOH afforded the hydroxamic acid analogues **14** and **15**. The phenolic carboxyl group could be replaced with a primary amide function by the same methodology. The ortho benzyl ester **4** was alkylated with 2-bromoacetamide to afford compound **16**. Boc deprotection with TFA and coupling with Boc-L-phenylalanine under standard carbodiimide conditions produced compound **17**. Final hydrogenation generated pure primary amide **18**.

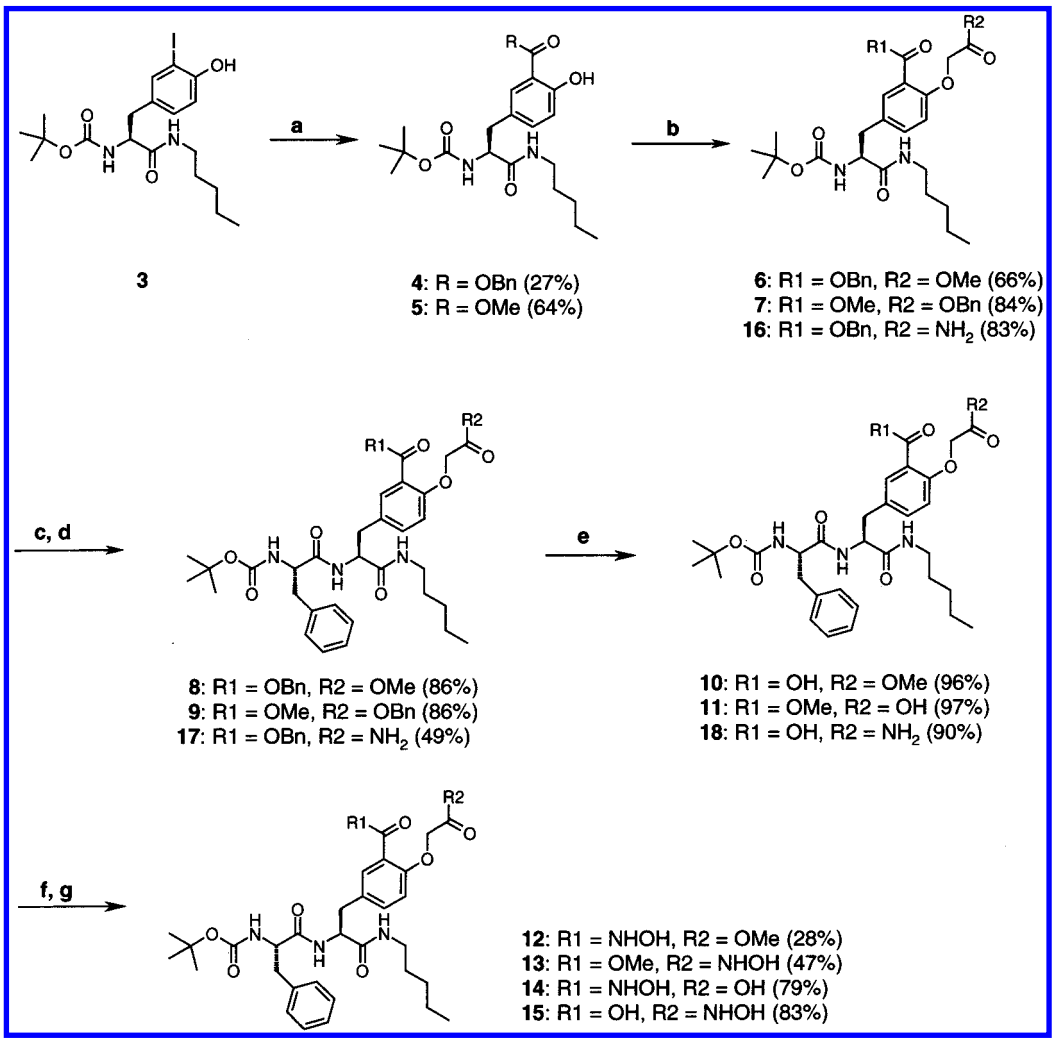
An interesting carboxylic acid isostere is the 3-hydroxyisoxazole group.[24] 3-Hydroxy-isoxazole can be synthesized from an acetylenic ester by reaction with hydroxylamine.[25] Introduction of the acetylenic ester was performed on the *O*-alkylated intermediate **19** using ethyl propiolate and copper(I) oxide as catalyst, which afforded compound **20** (Scheme 2).[26] Deprotection of the Boc group and coupling with Boc-L-phenylalanine under standard carbodiimide conditions generated compound **21**. Treatment of **21** with hydroxylamine afforded a mixture of the 3-hydroxyisoxazole compound and the corresponding hydroxamic acid analogues. Separation by reversed phase high-performance liquid chromatograph (HPLC) furnished pure target compound **22**. Hydrolysis of **21** with aqueous LiOH afforded the acetylenic analogue **23**, which was hydrogenated to generate the saturated analogue **24**.

Introduction of the lipophilic carboxylic acid bioisostere tetrazole group at both positions is shown in Schemes 3 and 4. Palladium-catalyzed cyanation reaction between compound **3** and zinc cyanide produced the nitrile analogue **25** in modest yield. Reported procedures describe the use of sodium cyanide and the cocatalyst CuI,[27] although the use of these conditions did not improve the yield for this reaction, probably because of the electronic effect of the ortho hydroxy group, which has a negative effect on the palladium coupling. Alkylation with methyl bromoacetate afforded compound **26**, which could be Boc deprotected and coupled with Boc-L-phenylalanine to generate compound **27**. Heating the nitrile analogue **27** with trimethylsilyl azide in toluene in the presence of catalytic amounts of dibutyltin oxide[28] gave the tetrazole analogue **28** in low but readily available yield. The low yield of this reaction is mostly due to unreacted starting material, which was the only detected side product. Hydrolysis with aqueous LiOH afforded the target compound **29**.

By similar methodology, the tetrazole group could be introduced at the phenolic position, as outlined in Scheme 4. Alkylation of the ortho methyl ester compound **5** with 2-bromoacetonitrile afforded the nitrile analogue **30**. Heating with trimethylsilyl azide in toluene, in the presence of catalytic amounts of dibutyltin oxide, afforded compound **31**. The subsequent steps were performed as previously described to generate the target compound **33**.

The nitrile analogues of the succinic acid analogue **1** could be prepared by a similar methodology as described in Schemes 3 and 4. Thus, starting from the nitrile

Scheme 1[a]



[a] Reagents: (a) CO(g), $Pd(OAc)_2$, DPPF, Et_3N, methanol or benzylalcohol, DMF, 80 °C. (b) Methyl 2-bromoacetate or benzyl 2-bromoacetate or 2-bromoacetamide, K_2CO_3, acetone, 50 °C. (c) TFA, CH_2Cl_2. (d) Boc-L-Phe-OH, EDC, HOBT, CH_2Cl_2. (e) H_2, 10% Pd/C, MeOH. (f) NH_2OH HCl, CDI, DMF, 80 °C. (g) LiOH, THF.

intermediates **26** and **30**, respectively, Boc deprotection and acylation with succinic anhydride and the subsequent hydrolysis with LiOH afforded the corresponding mononitrile analogues **34** and **35** (Table 2).

An analogue where the carboxylic acid in the phenolic position of **1** was replaced by a hydroxyl group could be prepared as outlined in Scheme 5. Initial attempts to alkylate the phenolic position of **5** by refluxing with 2-bromoethanol in the precence of base failed, and only starting materials could be recovered. The same reaction was performed in a tightly sealed Pyrex tube (Heck vial), as ethyleneoxide is probably formed during the reaction, which gave the seven-membered lactone analogue **36**. Similar conditions starting from the benzylester **4** gave the same product, which supports the structure of the intermediate lactone. Boc deprotection and the subsequent acylation with succinic anhydride formed the lactone succinic acid analogue **37**, which could be transferred to the hydroxyl analogue **38** by hydrolysis using LiOH as shown in Scheme 5.

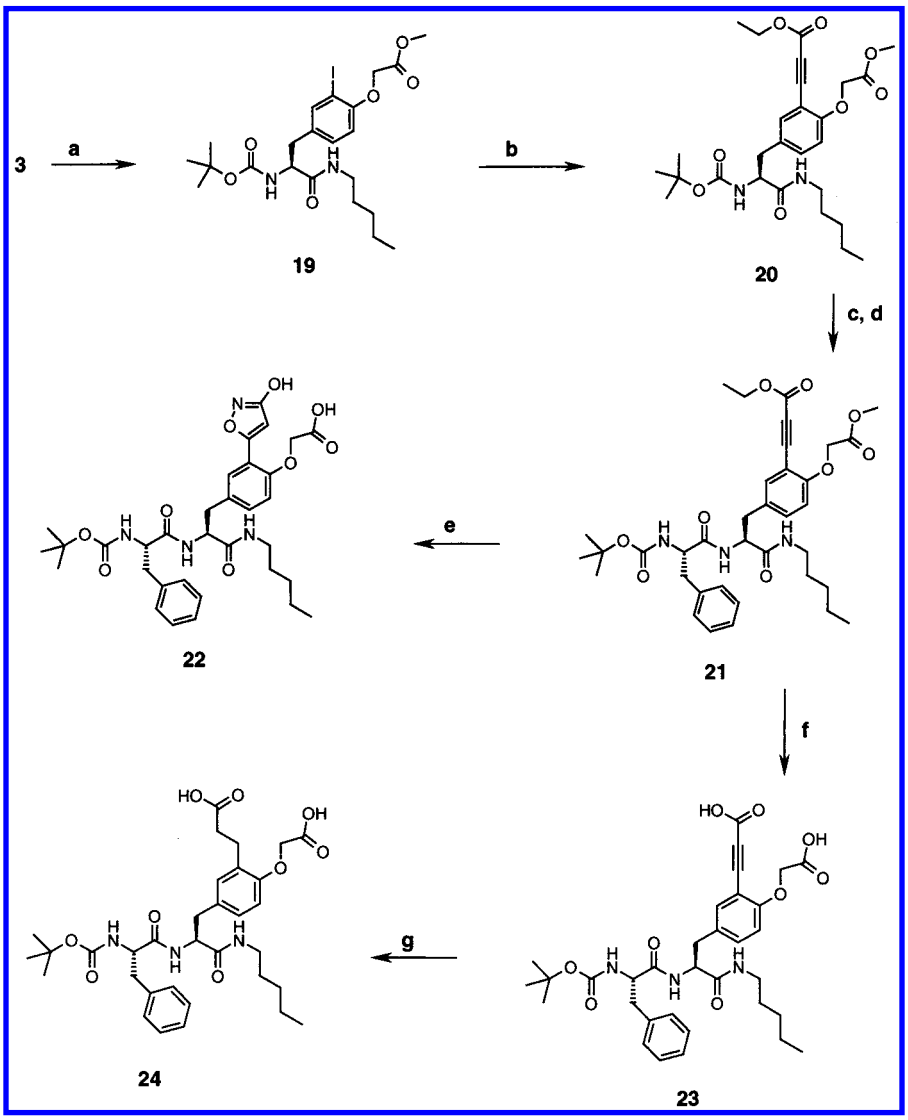
A methyl keto analogue of compound **1** could be prepared as outlined in Scheme 6. Palladium-catalyzed cross-coupling reaction between the aryl halide **3** and butyl vinyl ether and the subsequent hydrolysis of the resulting enol ether generated the methyl ketone **39**.[29] Compound **39** could be transformed to the succinic acid analogue **42** as previously described.

Results and Discussion

Competitive inhibition of PTP1B by the compounds was measured using pNPP as substrate, as previously described.[19] For the most active compounds, inhibition kinetics was determined, and from plots of V vs S at various concentrations of inhibitor, K_i values were computed using the direct linear method of Cornish−Bowden. Compounds were also tested for inhibition of other recombinant, purified PTP enzymes (LAR and SHP-2) as an intitial assessment of their specificity. Results are summarized in Tables 1 and 2. Activity of compounds against TC-PTP, the known PTP that is structurally most similar to PTP1B, was also determined. For clarity, only inhibition data for LAR and SHP-2 are presented here. In general, however, none exhibited significant selectivity between PTP1B and TC-PTP.

The monoester compounds **10** and **11**, and also the primary amide **18**, showed little or no inhibition of PTP1B. This verifies earlier findings that an acidic function at both positions is required for activity.[20] This is further supported by the inhibition exhibited by the

Scheme 2[a]



[a] Reagents: (a) Methyl bromoacetate, K_2CO_3, acetone, 50 °C (89%). (b) Ethyl propiolate, Cu_2O, DMF, 110 °C (47%). (c) TFA, CH_2Cl_2. (d) Boc-Phe-OH, HOBT, EDC, CH_2Cl_2 (84%). (e) NH_2OH HCl, NaOH, ethanol/THF (19%). (f) LiOH, THF (99%). (g) H_2, Pd/C, MeOH (98%).

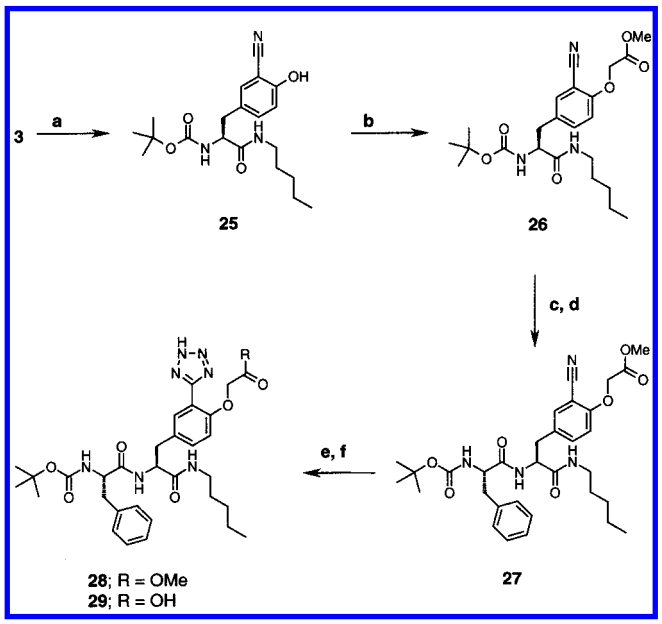
analogues of compound **1** shown in Table 2. The nitrile analogues **34** and **35**, and also the keto and hydroxy analogues **38** and **42**, are all inactive as inhibitors of PTP1B.

Extension of the ortho carboxylic acid group by two carbons (propynoic acid **23** and propanoic acid **24**) resulted in loss of inhibitory activity. This is most probably due to steric effects in the active site cleft. Interestingly, the ortho hydroxamic acid derivative **14** showed modest activity as a competitive inhibitor of PTP1B, whereas compound **15**, with hydroxamic acid at the phenolic position, was nearly inactive. This indicates that the ortho position apparently accommodates carboxyl replacements more easily.

The highlight of this work was the discovery of the ortho tetrazole analogue **29**, which was equipotent to the dicarboxylic acid derivative **1** as a PTP1B inhibitor (Table 1). This is the first monocarboxylic acid analogue in this series with high affinity for the PTP1B enzyme. The analogous derivative, with the tetrazole group at the phenolic position (**33**), could not be accommodated by the active site cleft and was nearly inactive. 3-Hydroxyisoxazole has been reported to be a good carboxylic acid bioisostere group.[24] However, the ortho 3-hydroxyisoxazole analogue **22** was inactive as a competitive inhibitor of PTP1B.

Tetrazole **29** indicated some increased permeability into Caco-2 cells[30] when compared to the dicarboxylic acid analogues, even though the values are still in the low permeability range. Compound **29** was thus tested for its effects on insulin-stimulated 2-deoxyglucose (2-DOG) uptake into intact cells (L6 myocytes). In the presence of insulin at a concentration (10 nM) that supported half-maximal 2-DOG uptake by L6 myocytes, compound **29** (100 μM) showed modest cell activity by augmenting insulin-stimulated uptake of 2-DOG to 147 $\pm$ 8% of that of the vehicle (0.1% dimethyl sulfoxide (DMSO)) control. However, basal uptake (without insulin) in the presence of compound **29** was 99 $\pm$ 2% that of the control cells.[31] Because of the polar properties of these compound series, we have not been able to demonstrate reproducible cell activities with previous tested analogues, the exception being prodrug tri- and diesters, which are likely to be hydrolyzed intracellu-

Scheme 3[a]



[a] Reagents: (a) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 80 °C (38%). (b) Methyl bromoacetate, K_2CO_3, acetone, 50 °C (94%). (c) TFA, CH_2Cl_2. (d) Boc-L-Phe-OH, EDC, HOBT, CH_2Cl_2 (84%). (e) TMS-N_3, Bu_2SnO (catalytic), toluene, 110 °C (19%). (f) LiOH, THF (90%).

larly.[20] The modest cell activity of compound **29** is consistent with the evidence that this analogue has increased permeability properties and is able to penetrate into cells to exert its effects. It also supports the hypothesis that PTP1B inhibitors can be developed into antihyperglycemic therapy.

Structure Solution of PTP1B−Compound 29 Complex. The mode of binding of the ortho tetrazole analogue **29** was determined by solving an X-ray cocrystal structure with a C-terminal truncated form of PTP1B (Figures 1 and 2). As with the crystal structure of PTP1B complexed with **2**,[20] only one of the two protein molecules in the asymmetric unit was observed to have ligand bound. The binding site of the second PTP1B molecule was effectively blocked by crystal contacts from a symmetry-related PTP1B molecule. In the binding site that does contain bound ligand, however, the overall structure of the PTP1B−compound **29** complex is very similar to that previously reported for **2**[20] (Figures 1 and 2). This was not unexpected though as the two compounds differ only in the ortho substituent of the tyrosine headgroup with the replacement of a carboxylic acid by the tetrazole moiety.

As observed with **2**, the binding of **29** is accompanied by closure of the mobile WPD loop (comprised of residues 179−189) of the PTP1B catalytic site. The peptide backbone portion of both bound inhibitors forms an extended β-strand conformation closely resembling the conformation of peptidic substrates.[32] Two conserved hydrogen bonds are maintained between the side chain of Asp48 and the main chain nitrogens on either side of the central tyrosine phosphate mimic (P and P + 1 positions) and a third between the main chain nitrogen of Arg47 and a main chain carbonyl of the inhibitor at the P − 2 position. The phenyl ring of the phosphotyrosine (pTyr) mimic also makes hydrophobic packing interactions with the side chains of Tyr46, Val49, Ala217, Ile219, Gln262, and Phe181. The P − 1 phenyl ring of both compounds **2** and **29** makes hydrophobic interactions with the side chains of Arg47 and Asp48. This substituent is also observed to make a crystal contact with the side chain of Phe280 from the neighboring PTP1B molecule. In addition, the C-terminal pentyl chain (P + 1) of both compounds lies in a shallow hydrophobic pocket and forms hydrophobic contacts with the side chains of Val49, Ile219, and Gln262 (Figure 2).

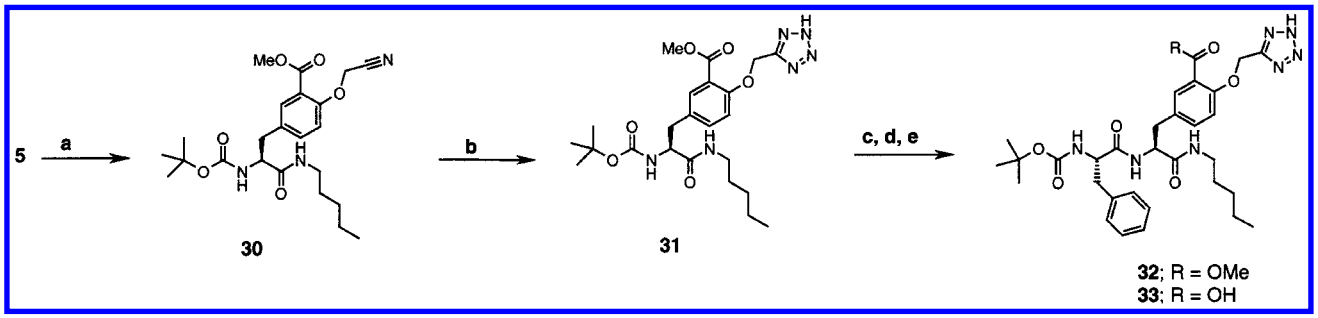
The terminal O-methylcarboxy of the tyrosine headgroup makes a series of four direct hydrogen bonds to the main chain nitrogen of Phe182, the side chain amide of Gln266, the side chain NE1, and the main chain nitrogen of Arg221. In addition, two water molecules are hydrogen-bonded to the ether oxygen. These waters are positioned similarly to the phosphate oxygen atoms of the peptide substrate crystal structures and make similar hydrogen bond interactions with the backbone amides of Ser216, Ala217, Ile219, and Gly220.[32]

The ortho tetrazole substituent on the tyrosine headgroup is directed toward Lys120, but it is unable to make the hydrogen bond observed by the ortho carboxylate of compound **2**. The loss of this hydrogen bond, however, is at least partially compensated by more extensive hydrophobic and van der Waals interactions with Phe182, Tyr46, Asp181, Ser216, and Lys120.

Conclusions

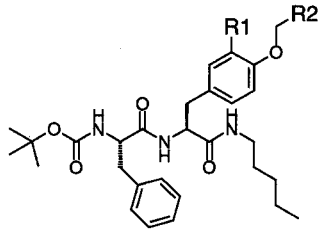
Modification of the tyrosine head moiety of **1** and **2** by the introduction of carboxylic acid bioisosteres has verified the importance of acidic functionality at both positions. Nonacidic replacements such as ester, alcohol, keto, and carboxamide gave inactive compounds. Replacement by less acidic groups with greater lipophilicity resulted in compounds with varying degrees of

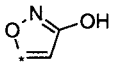
Scheme 4[a]



[a] Reagents: (a) Bromoacetonitrile, K_2CO_3, acetone, 16 h (93%). (b) TMS-N_3, Bu_2SnO (catalytic), toluene, 110 °C (37%). (c) TFA, CH_2Cl_2. (d) Boc-L-Phe-OH, EDC, HOBT, CH_2Cl_2 (32%). (e) LiOH, THF (99%).

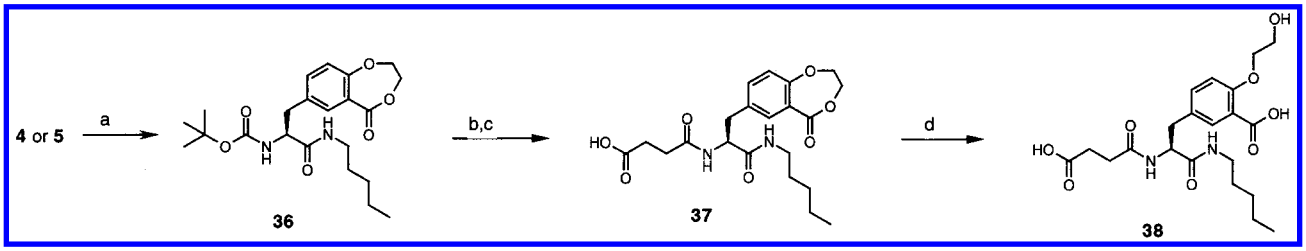
Table 1. Tyrosine Carboxyl Replacements of the N-Terminal Boc-Phe Analogue **2**



			% Inhibition of Phosphatases (Ki)			
Cmpd.	R1	R2	PTP1B 100, 10, 1 μM		LAR 100 μM	SHP-2 100 μM
2[a]	COOH	COOH	97, 78, 30	(2.0 μM)	0	2
10	$COOCH_3$	COOH	4, 2, 3		0	0
11	COOH	$COOCH_3$	25, 5, 3		0	0
14	CONHOH	COOH	54, 13, 5		3	6
15	COOH	CONHOH	30, 8, 6		1	4
18	COOH	$CONH_2$	0, 0, 0		0	0
22		COOH	9, 1, 1		0	nd
23	CCCOOH	COOH	16, 3, 1		1	1
24	CH_2CH_2COOH	COOH	18, 4, 3		13	1
29		COOH	97, 77, 27	(2.0 μM)	0	1
33	COOH		17, 3, 1		0	5

[a] Described in ref 19.

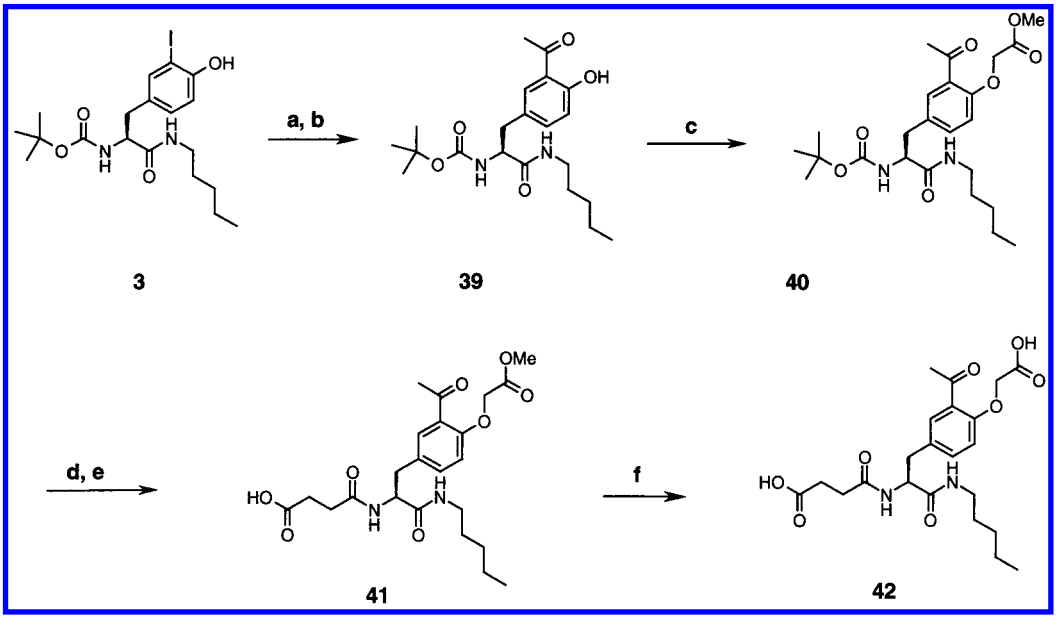
Scheme 5[a]



[a] Reagents: (a) 2-Bromoethanol, K_2CO_3, DMF, 80 °C, sealed Heck vial (21%). (b) TFA, CH_2Cl_2. (c) Succinic acid anhydride, TEA, CH_2Cl_2 (42%). (d) LiOH, THF (75%).

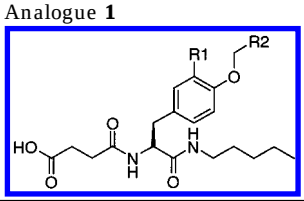
activity. Modification of the carboxylic acid appended to the phenol of the tyrosine headgroup resulted in inactive compounds, whereas the intermediate activity of the ortho hydroxamic acid analogue **14** indicated that

Scheme 6[a]



[a] Reagents: (a) Butyl vinyl ether, $Pd(OAc)_2$, DPPP, TlOAc, DMF, 90 °C, 16 h. (b) HCl, H_2O (41%). (c) Methyl 2-bromoacetate, K_2CO_3, acetone (36%). (d) TFA, CH_2Cl_2. (e) Succinic anhydride, Et_3N, CH_2Cl_2 (64%). (f) LiOH, THF/MeOH/H_2O (59%).

Table 2. Tyrosine Carboxyl Replacements of the N-Terminal Succinamic Acid Analogue **1**



			% inhibition of phosphatases (K_i)[a]		
compd	R1	R2	PTP1B	LAR	SHP-2
1[b]	COOH	COOH	87 (6.5 μM)	0	5
34	CN	COOH	4	0	0
35	COOH	CN	3	0	0
38	COOH	CH_2OH	4	0	0
37	(lactone) $COOCH_2CH_2$		1	0	0
42	$COCH_3$	COOH	8[c]	−2	1

[a] Assayed at 100 μM. [b] Described in refs 18 and 19. [c] Inhibition measured on PTP1 (rat homologue of PTP1B).

the ortho position might tolerate replacements more easily. This was confirmed by the important discovery of the ortho tetrazole analogue **29**, which was equipotent to the dicarboxylic acid analogue **2**. Solution of the X-ray cocrystal structure of the ortho tetrazole analogue **29** bound to PTP1B indeed revealed that the tetrazole moiety is well-accommodated in the active site and binds in a similar fashion to the previously reported ortho carboxylate analogue **2**. Of particular importance, this new monocarboxylic acid analogue revealed significantly higher Caco-2 cell permeability as compared to all previous compounds. The increased permeability properties of compound **29** may account for its modest augmentation of insulin-stimulated 2-DOG uptake into L6 myocytes. This result represents the first positive cell activity data from nonprodrug analogues of this compound class and demonstrates the potential of these analogues to be developed into drug therapy for type 2 diabetes.

Experimental Section

Chemistry. General Comments. All experiments were carried out under an N_2 atmosphere, except the hydrogenation and carbonylation reactions. Melting points were determined in open glass capillaries on a Gallenkamp apparatus and were not corrected. ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR were recorded on a Bruker Advance DPX 400 spectrometer at 400.1 and 100.6 MHz, respectively, or on a Bruker DRX 500 at 500 MHz and 125.7 MHz, respectively. ^1H NMR and ^{13}C NMR spectra were referenced to internal tetramethylsilane. IR spectra were recorded on a Perkin-Elmer Spectrum 1000 FT-IR spectrophotometer. Ionspray mass spectrometry (MS) spectra were obtained on a Perkin-Elmer API 150EX mass spectrometer or a Micromass Platform-LCZ instrument. Preparative HPLC were performed on a Gilson HPLC 305, column: YMC-Pack AQ (50 mm × 4.6 mm; 2 μM 120 Å 3); eluent: A-MilliQ/0.1% TFA B−CH_3CN. Analytical HPLC were performed on a Shimadzu LC-6A liquid chromatograph using HiChrom Nucleosil C-18 column (HCl; size, 100 ,, × 4.6 mm) and Jour C8 precolumn; mobile phase was CH_3CN/water (0.1% TFA) at 1 mL/min. Elementary analyses were performed by Mikro Kemi AB, Uppsala, Sweden.

Benzyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-hydroxybenzoate (4). Triethylamine (1.71 mL, 12.5 mmol) and benzyl alcohol (6.45 mL, 62 mmol) were added to a suspension of **3** (2.97 g, 6.23 mmol), palladium(II) acetate (42 mg, 0.19 mmol), and 1,1′-bis(diphenylphosphino)ferrocene (DPPF, 207 mg, 0.37 mmol) in dimethyl formamide (DMF; 15 mL). The mixture was saturated with CO (1 atm) and stirred at 70 °C for 16 h. The mixture was allowed to reach room temperature and extracted with EtOAc (40 mL). The organic layer was washed with 10% aqueous HCl (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_2, EtOAc/*n*-hexane 1:2), which furnished 5.7 g of a yellow oil. This crude material still contained some benzyl alcohol. Crystallization in EtOAc/*n*-hexane gave 0.82 g (27%) of pure **4** as a white solid. ^1H NMR 400 MHz ($CDCl_3$): δ 0.84 (t, 3H, J = 7.1, 14.4), 1.13 (m, 2H), 1.23 (m, 2H,), 1.35 (m, 2H), 1.39 (s, 9H), 2.95 (d, 2H, J = 6.7), 3.11 (m, 2H), 4.18 (m, 1H), 5.07 (br m, 1H), 5.37 (d, 2H, J = 1,7), 5.77 (br m, 1H), 6.91 (d, 1H, J = 8.5), 7.30 (dd, 1H, J = 2.2, 8.5), 7.35−7.47 (m, 5H), 7.69 (d, 1H, J = 2.2), 10.69. ^{13}C NMR ($CDCl_3$): δ 13.9, 22.3, 28.2, 28.4, 28.9, 29.0, 37.7, 39.4, 56.8, 67.1, 80.3, 112.2, 117.9, 127.5, 128.5, 128.6, 128.7, 130.3, 135.1, 136.9, 155.8,

160.7, 169.8, 170.7. MS (ESI) *m*/*z* 485 (M + H). Anal. ($C_{27}H_{36}N_2O_6$) C, H, N.

Methyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-hydroxybenzoate (5). Triethylamine (0.61 mL, 4.41 mmol) was added to a stirring suspension of **3** (1.05 g, 2.20 mmol), palladium(II) acetate (14 mg, 0.066 mmol), and DPPF (73 mg, 0.13 mmol) in DMF/MeOH 4:1 (5 mL). A carbon monoxide atmosphere was established in the reaction vessel, and the mixture was stirred at 70 °C for 16 h. After the mixture was cooled to ambient temperature, the mixture was extracted with EtOAc (5 mL), and the organic layer was washed with 10% aqueous HCl (2 × 2 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc/*n*-hexane 1:2), which furnished 0.54 g (60%) of **5** as a white solid. ^{1}H NMR 500 MHz ($CDCl_3$): δ 0.86 (t, 3H, J = 7.2, 14.6), 1.17 (m, 2H), 1.25 (m, 2H), 1.36 (m, 2H), 1.41 (s, 9H), 2.97 (d, 2H, J = 7.2), 3.12−3.21 (m, 2H), 3.92 (s, 3H), 4.21 (dd, 1H, J = 14.7, 7.2), 5.11 (br s, 1H), 5.81 (br m, 1H), 6.91 (d, 1H, J = 8.5), 7.30 (dd, 1H, J = 8.5, 2.1), 7.67 (d, 1H, J = 2.1). ^{13}C NMR ($CDCl_3$): δ 13.86, 22.24, 28.26 (3C), 28.90, 29.05, 37.77, 39.47, 52.23, 54.69, 80.37, 112.25, 117.82, 127.54, 130.34 (2C), 136.76, 160.56, 170.32, 170.74. Anal. ($C_{21}H_{32}O_6N_2$) C, H.

General Alkylation Procedure. The general procedure for alkylation of the phenolic position is exemplified by the preparation of compound **6**. Methyl bromoacetate (0.35 mL, 3.75 mmol) and freshly ground K_2CO_3 (0.52 g, 3.75 mmol) were added to a solution of **4** (0.61 g, 1.25 mmol) in acetone (15 mL). The mixture was stirred at 50 °C overnight. After the mixture was cooled to ambient temperature, H_2O (10 mL) was added and the mixture was extracted with EtOAc (10 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (SiO_2, EtOAc/*n*-hexane 1:1).

Benzyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)benzoate (6). The reaction was carried out with methyl 2-bromoacetate (0.35 mL, 3.75 mmol) and **4** (0.61 g, 1.25 mmol), yielding 0.46 g (66%) of **6** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.87 (t, 3H, J = 7.1, 14.3), 1.17−1.40 (m, 6H), 1.35 (s, 9H), 2.79 (dd, 1H, J = 8.4, 13.6), 2.98−3.14 (m, 3H), 3.74 (s, 3H), 4.19 (m, 1H), 4.75 (s, 2H), 5.33 (s, 2H), 6.95 (d, 1H, J = 8.5), 7.32−7.39 (m, 5H), 7.46 (dd, 1H, J = 1.9, 8.5), 7.67 (d, 1H, J = 1.9). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.9, 30.2, 30.4, 38.7, 40.6, 52.9, 57.7, 67.4, 68.1, 80.9, 115.8, 122.2, 129.5, 129.5, 129.9, 132.2, 133.7, 136.0, 137.9, 157.8, 158.1, 167.8, 171.1, 174.0. MS (ESI) *m*/*z* 555 (M − H). Anal. ($C_{30}H_{40}N_2O_8$) C, H, N.

Methyl 2-[2-(Benzyloxy)-2-oxoethoxy]-5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-benzoate (7). The reaction was carried out with benzyl 2-bromoacetate (0.84 mL, 5.3 mmol) and **5** (0.72 g, 1.77 mmol) according to the general alkylation procedure, yielding 0.82 g (84%) of **7** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.89 (t, 3H, J = 6.8, 13.9), 1.22 (m, 2H), 1.29 (m, 2H), 1.37 (s, 9H), 1.39 (m, 2H), 2.81 (dd, 1H, J = 8.5, 13.3), 3.00 (dd, 1H, J = 7.4, 13.3), 3.07−3.15 (m, 2H), 3.82 (s, 3H), 4.20 (m, 1H), 4.79 (s, 2H), 5.21 (s, 2H), 6.91 (d, 1H, J = 8.5), 7.31−7.35 (m, 6H), 7.66 (s, 1H). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.9, 30.3, 30.4, 38.7, 40.7, 52.8, 57.7, 67.5, 68.2, 80.9, 115.6, 122.1, 129.6, 129.7, 129.9, 132.2, 133.7, 135.8, 137.3, 158.0, 168.4, 170.5, 174.1. MS (ESI) *m*/*z* 555 (M − H). Anal. ($C_{30}H_{40}N_2O_8$) C, H, N.

General Boc Deprotection and Carbodiimide Coupling Procedure. The general procedure for Boc deprotection and carbodiimide coupling is exemplified by the preparation of compound **8**. Trifluoroacetic acid (0.90 mL) was carefully added to a solution of **6** (0.44 g, 0.78 mmol) in CH_2Cl_2 (8 mL) at 0 °C. The mixture was stirred for 4 h allowing the solution to warm to ambient temperature. The volatiles were removed in vacuo, and the residue was partitioned between EtOAc (15 mL) and saturated aqueous $NaHCO_3$ (2 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated, which gave 0.35 g (98%) of the crude amine as a yellowish oil. The amine was dissolved in CH_2Cl_2 (7 mL) and cooled with ice. Boc-L-phenylalanine (0.20 g, 0.77 mmol), 1-hydroxybenzotriazole (0.10 g, 0.77 mmol), and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.15 g, 0.77 mmol) were added to the solution, which was then stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with saturated aqueous $NaHCO_3$ (5 mL), brine (5 mL), and 10% aqueous HCl (5 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (SiO_2, EtOAc).

Benzyl 5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)benzoate (8). Compound **8** was prepared according to the general procedure for Boc deprotection and carbodiimide coupling, yielding 0.46 g (86%) as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.86 (t, 3H, J = 7.2, 14.5), 1.18 (m, 2H), 1.24 (m, 2H), 1.34 (s, 9H), 1.38 (m, 2H), 2.72 (dd, 1H, J = 9.4, 13.8), 2.89−3.11 (m, 3H), 3.71 (s, 3H), 4.22 (m, 1H), 4.49 (m, 1H), 4.74 (s, 2H), 5.31 (s, 2H), 6.94 (d, 1H, J = 8.6), 7.14−7.46 (m, 6H), 7.65 (d, 1H, J = 1.7). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.6, 28.9, 30.2, 30.4, 38.3, 39.3, 40.7, 52.9, 56.1, 58.0, 67.4, 68.1, 81.1, 115.8, 122.3, 128.0, 129.5, 129.5, 129.7, 129.9, 130.6, 131.8, 133.8, 136.0, 137.9, 138.7, 158.2, 167.8, 171.0, 172.7, 174.3. MS (ESI) *m*/*z* 702 (M − H). Anal. ($C_{39}H_{49}N_3O_9$) C, H, N.

Methyl 2-[2-(Benzyloxy)-2-oxoethoxy]-5-[(2*S*)-2-({(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoyl}-amino)-3-oxo-3-(pentylamino)propyl]benzoate (9). The reaction was carried out with **7** (0.78 g, 1.41 mmol) according to the general Boc deprotection and carbodiimide reaction procedure, yielding 0.85 g (86%) of **9** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.4), 1.18 (m, 2H), 1.28 (m, 2H), 1.34 (s, 9H), 1.37 (m, 2H), 2.74 (dd, 1H, J = 9.4, 13.8), 2.93 (dd, 1H, J = 7.5, 13.8), 2.97−3.05 (m, 3H), 3.12 (m, 1H), 3.82 (s, 3H), 4.23 (m, 1H), 4.50 (m, 1H), 4.79 (s, 2H), 5.18 (s, 2H), 6.69 (br d, 0.6H), 6.91 (d, 1H, J = 8.6). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.7, 28.9, 30.2, 30.4, 38.3, 39.3, 40.8, 40.9, 52.9, 56.1, 58.0, 67.4, 68.2, 81.1, 115.7, 122.2, 128.0, 129.7, 129.7, 129.9, 130.6, 131.7, 133.8, 135.9, 137.3, 138.8, 158.1, 168.4, 170.4, 172.7, 172.8, 174.3. MS (ESI) *m*/*z* 702 (M − H). Anal. ($C_{39}H_{49}N_3O_9$) C, H, N.

5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)benzoic Acid (10). A mixture of **8** (0.41 g, 0.58 mmol) and 10% Pd/C (80 mg) in methanol (25 mL) was hydrogenated at atmospheric pressure for 2 h. The mixture was filtered through Celite, and the solvent was removed under reduced pressure to afford 0.34 g (96%) of **10** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.89 (t, 3H, J = 7.1, 14.4), 1.19−1.42 (m, 6H), 1.36 (s, 9H), 2.73 (dd, 1H, J = 9.5, 13.8), 2.93−3.07 (m, 3H), 3.14 (m, 1H), 3.75 (s, 2H), 4.23 (m, 1H), 4.51 (br m, 1H), 4.81 (s, 2H), 6.96 (d, 1H, J = 8.5), 7.17−7.27 (m, 5H), 7.35 (dd, 1H, J = 1.8, 8.5), 7.72 (d, 1H, J = 1.8), 7.84 (br m, 0.5H), 7.98 (br m, 0.2H). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.9, 30.2, 30.2, 30.4, 38.3, 39.3, 40.8, 53.0, 56.1, 58.0, 67.4, 81.1, 115.6, 122.7, 128.0, 129.7, 130.6, 132.0, 134.2, 135.9, 138.8, 157.9, 158.2, 169.7, 171.2, 172.7, 172.8, 174.3. MS (ESI) *m*/*z* 612 (M − H). Anal. ($C_{32}H_{43}N_3O_9$) C, H, N.

2-[4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(methoxycarbonyl)phenoxy]acetic Acid (11). A mixture of **9** (0.66 g, 0.94 mmol) and 10% Pd/C (100 mg) in methanol (30 mL) was hydrogenated at atmospheric pressure for 2 h. The mixture was filtered through Celite, and the solvent was removed under reduced pressure to afford 0.57 g (97%) of **11** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.89 (t, 3H, J = 7.1, 14.4), 1.21 (m, 2H), 1.29 (m, 2H), 1.35 (s, 9H), 1.37 (m, 2H), 2.75 (dd, 1H, J = 9.3, 13.8), 2.94 (dd, 1H, J = 7.4, 13.8), 2.98−3.05 (m, 3H), 3.13 (m, 1H), 3.86 (s, 3H), 4.24 (m, 1H), 4.50 (m, 1H), 4.69 (s, 2H), 6.95 (d, 1H, J = 8.6), 7.17−7.27 (m, 5H), 7.35 (dd, 1H, J = 1.5, 8.6), 7.64 (d, 1H, J = 1.5), 7.83 (br m, 1H), 7.98 (br d, 0.6H). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.7, 28.9, 30.2, 30.4, 38.3, 39.3, 40.7, 40.9, 52.9, 56.2, 58.0, 67.5, 81.1, 115.8, 121.9, 128.0, 129.7, 130.6, 131.6, 133.8,

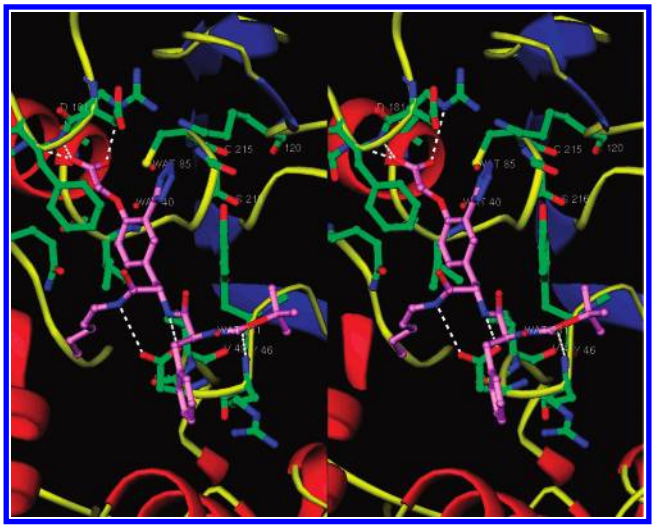


Figure 1. Stereo representation of the crystal structure (2.3 Å resolution) of **29** bound to PTP1B. The enzyme backbone is represented as a ribbon diagram with key residues presented in detail and labeled. Heteroatoms are colored by type (carbon, green for PTP1B and purple for **29**; oxygen, red; nitrogen, blue; sulfur, yellow). Selected hydrogen-bonding interactions with the enzyme are depicted with dashed lines.

136.1, 138.8, 158.1, 158.4, 168.5, 172.7, 172.8, 174.3. MS (ESI) *m*/*z* 612 (M − H). Anal. ($C_{32}H_{43}N_3O_9$) C, H, N.

General Procedure for the Introduction of Hydroxamic Acid. The general procedure for introducing hydroxamic acid is exemplified by the preparation of **12**. To a solution of **10** (116 mg, 0.19 mmol) in THF/DMF (2.5:0.2 mL) was added CDI (61 mg, 0.38 mmol), and the mixture was refluxed at 80 °C for 1 h. The mixture was cooled to ambient temperature, and hydroxylamine hydrochloride (39 mg, 0.57 mmol) was added. The reaction mixture was refluxed at 80 °C for 4 h. After the mixture was cooled to room temperature, the mixture was partitioned between EtOAc (3 mL) and 3 M aqueous HCl (3 mL), and the organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (SiO_2, 5% MeOH in CH_2Cl_2) and further purified by crystallization in EtOAc.

Methyl 2-{4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-[(hydroxyamino)carbonyl]phenoxy}acetate (12). Compound **12** was repared according to the general procedure for introducing hydroxamic acid, yielding 34 mg (28%) of **12** as a white solid; mp 165.4−166.9 °C. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.89 (t, 3H, J = 7.1, 14.4), 1.20 (m, 2H), 1.29 (m, 2H), 1.35 (s, 9H), 1.38 (m, 2H), 2.70 (dd, 1H, J = 9.4, 13.3), 2.91−3.17 (m, 5H), 3.81 (s, 3H), 4.21 (m, 1H), 4.54 (m, 1H), 4.86 (s, 2H, obscured by solvent peak), 6.98 (d, 1H, J = 8.1), 7.16−7.27 (m, 5H), 7.34 (br d, 1H, J = 8.1), 7.84 (br s, 1H). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.9, 30.2, 30.4, 38.4, 39.2, 40.7, 56.1, 58.1, 67.3, 81.1, 114.7, 121.9, 128.0, 129.7, 130.6, 132.5, 133.4, 135.3, 135.8, 138.8, 156.2, 158.1, 165.7, 171.3, 172.8, 174.6. MS (ESI) *m*/*z* 627 (M−H). Anal. ($C_{32}H_{44}N_4O_9 \cdot 1/2H_2O$): C, H, N.

Methyl 5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-[2-(hydroxyamino)-2-oxoethoxy]benzoate (13). The reaction was carried out with **11** (103 mg, 0.17 mmol) according to the general procedure for introducing hydroxamic acid, although column chromatography (SiO_2, 5% MeOH in CH_2Cl_2) was sufficient to afford 46 mg (44%) of pure title compound **13** as a white solid; mp = 160.9−163.5 °C. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.5), 1.19 (m, 2H), 1.28 (m, 2H), 1.35 (s, 9H), 1.37 (m, 2H) 2.77 (dd, 1H, J = 9.2, 13.7), 2.93 (dd, 1H, J = 7.5), 2.98−3.06 (m, 3H), 3.13 (m, 1H), 3.90 (s, 3H), 4.22 (dd, 1H, J = 5.3, 9.1), 4.50 (app t, 1H), 4.66 (s, 2H), 7.06 (d, 1H, J = 8.5), 7.17−7.27 (m, 5H), 7.43 (dd, 1H, J = 1.8, 8.5), 7.75 (d, 1H, 1.8). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.9, 30.2, 30.4, 38.4, 39.3, 40.7, 53.1, 56.1, 58.0, 69.3, 81.1, 116.1, 120.9, 128.1, 129.7, 130.6, 132.2, 134.1, 136.9, 138.7, 158.4, 167.7, 168.0, 172.7. 174.3. MS (ESI) *m*/*z* 627 (M − H). Anal. ($C_{32}H_{44}N_4O_9$) C, H, N.

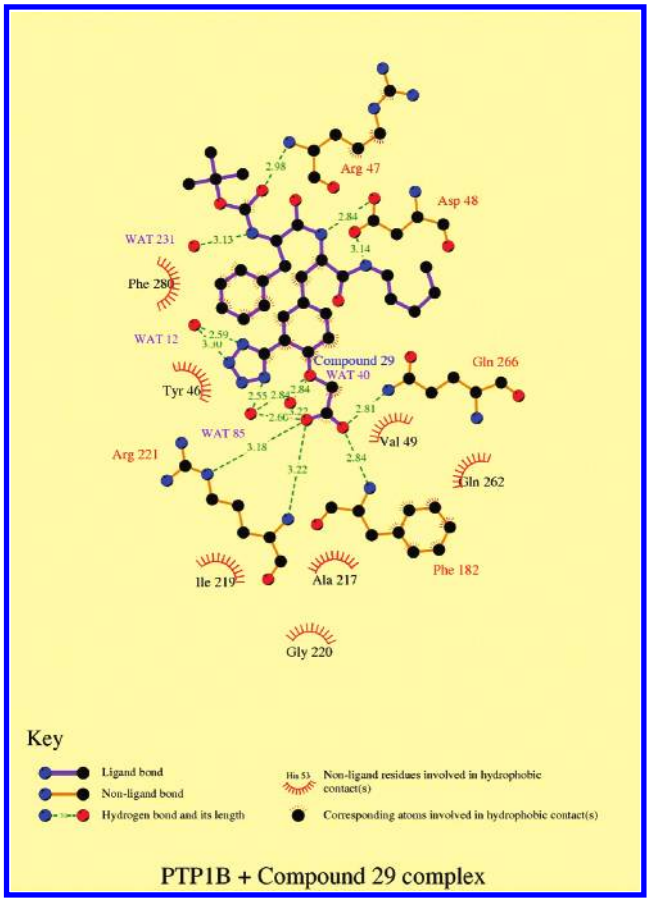


Figure 2. Schematic representation of the interactions between PTP1B and compound **29**. Green dotted lines represent hydrogen bonds or electrostatic interactions, and red dotted lines represent hydrophobic interactions. Atom colors are as follows: carbon, black; nitrogen, blue; oxygen, red; and sulfur, yellow. Distances are shown in Ångströms.

2-{4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-[(hydroxyamino)carbonyl]phenoxy}acetic Acid (14). To a solution of **12** (10 mg, 0.0165 mmol) in THF (200 μL) was added a 2.5 M aqueous solution of LiOH (9.9 μL, 0.0248 mmol), and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was then acidified with 3 M aqueous HCl and extracted with EtOAc (2 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford 8.0 mg (79%) of **14** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.5), 1.19 (m, 2H), 1.29 (m, 2H), 1.35 (s, 9H), 1.38 (m, 2H), 2.71 (dd, 1H, J = 9.5, 13.5), 2.94 (m, 1H), 2.98−3.07 (m, 3H), 3.12 (m, 2H), 4.22 (dd, 1H, J = 5.1, 9.5), 4.54 (m, 1H), 4.81 (s, 2H), 6.99 (d, 1H, J = 8.5), 7.17−7.27 (m, 5H), 7.35 (dd, 1H, J = 1.8, 8.5), 7.85 (d, 1H, J = 1.8). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.9, 30.2, 30.4, 38.4, 39.2, 40.7, 56.1, 58.0, 67.3, 81.1, 114.8, 121.9, 128.0, 129.7, 130.6, 132.4, 133.4, 135.4, 138.8, 156.3, 158.1, 165.7, 172.4, 172.8, 174.3. MS (ESI) 613 (M − H). Anal. ($C_{31}H_{42}N_4O_9 \cdot 1/2H_2O$) C, H, N.

5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-[2-(hydroxyamino)-2-oxoethoxy]benzoic Acid (15). To a solution of **13** (10 mg, 0.016 mmol) in THF (400 μL) was added a 2.5 M aqueous solution of LiOH (19 μL, 0.048 mmol), and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was then acidified with 3 M aqueous HCl and extracted with EtOAc (2 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford 8 mg (83%) of **15** as a

white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.5), 1.19 (m, 2H), 1.29 (m, 2H), 1.35 (s, 9H), 1.38 (m, 2H), 2.78 (dd, 1H), 2.95 (dd, 1H), 3.14 (m, 1H), 4.23 (dd, 1H, 5.2, 9.2), 4.51 (m, 1H), 4.68 (s, 2H), 7.04 (d, 1H, J = 8.5), 7.17−7.27 (m, 5H), 7.42 (dd, 1H, J = 2.1, 8.5), 7.78 (d, 1H, J = 2.1). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.4, 286, 29.9, 30.1, 38.1, 39.0, 40.5, 55.8, 57.7, 68.9, 80.9, 115.8, 121.2, 127.7, 129.4, 130.3, 131.9, 134.2, 136.4, 138.4, 158.2, 167.6, 169.0, 172.4, 172.5, 174.0. MS (ESI) m/z 613 (M − H). Anal. ($C_{31}H_{42}N_4O_9$·1/2H_2O) C, H, N.

Benzyl 2-(2-Amino-2-oxoethoxy)-5-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]benzoate (16). The reaction was carried out with compound **4** (295 mg, 0.61 mmol) and 2-bromoacetamide (168 mg, 1.22 mmol) according to the general alkylation procedure, yielding 273 mg (83%) of **16** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.4), 1.18 (m, 2H), 1.27 (m, 2H), 1.37 (s, 9H), 1.40 (m, 2H, partly obscured), 2.82 (dd, 1H, J = 8.4, 13.5), 3.00−3.09 (m, 2H), 3.13 (m, 1H), 3.85 (s, 0.5H), 4.21 (br m, 1H), 4.56 (s, 2H), 5.36 (s, 2H), 7.07 (d, 1H, J = 8.5), 7.36−7.49 (m, 6H), 7.81 (s, 1H). ^{13}C NMR (MeOH-d_4): δ 14.7, 23.8, 29.0, 30.4, 30.5, 38.9, 40.7, 57.8, 68.2, 69.1, 81.0, 115.7, 120.7, 129.8, 129.9, 130.1, 132.4, 134.2, 137.0, 137.8, 157.9, 158.4, 167.2, 174.0, 174.1. MS (ESI) m/z 542 (M + H). Anal. calcd for $C_{29}H_{39}N_3O_7$·2H_2O: C, 60.30; H, 6.80; N, 7.27. Found: C, 60.0; H, 6.6; N, 7.9.

Benzyl 2-(2-Amino-2-oxoethoxy)-5-[(2*S*)-2-({(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]benzoate (17). The reaction was carried out with **16** (72 mg, 0.13 mmol) according to the general procedure for Boc deprotection and carbodiimide coupling, yielding 44 mg (49%) of **17** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.85 (t, 3H, J = 7.2, 14.5), 1.13 (m, 2H), 1.23 (m, 2H), 1.34 (s and m, 9H and 2H), 2.78 (dd, 1H, J = 9.5, 13.5), 2.9−3.13 (m, 5H), 4.21 (dd, 1H), 4.49 (br t, 1H), 4.53 (s, 2H), 5.34 (s, 2H), 7.03 (d, 1H, J = 8.5), 7.15−7.26 (m, 5H), 7.33−7.46 (m, 6H), 7.78 (s, 1H). ^{13}C NMR (MeOH-d_4): δ 14.7, 23.8, 29.0, 30.3, 30.5, 38.5, 39.4, 40.8, 56.2, 58.1, 68.3, 69.1, 81.2,115.7, 120.8, 128.1, 129.8, 129.9, 130.1, 130.7, 131.9, 134.3, 137.1, 137.8, 138.8, 158.1, 158.5, 167.1, 172.7, 174.0, 174.4. MS (ESI) m/z 687 (M − H). Anal. ($C_{38}H_{48}N_4O_8$) C, H, N.

2-(2-Amino-2-oxoethoxy)-5-[(2*S*)-2-({(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]benzoic Acid (18). A mixture of **17** (38 mg, 0.056 mmol) and 10% Pd/C (12 mg) in methanol (2.2 mL) was hydrogenated at atmospheric pressure for 3 h. The mixture was filtered through Celite, and the solvent was removed under reduced pressure to afford 30 mg (90%) of **18** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.5), 1.19 (m, 2H), 1.29 (m, 2H), 1.35 (s, 9H), 1.39 (m, 2H, partly obscured), 2.73 (dd, 1H, J = 9.6, 13.6), 2.92−3.06 (m, 4H), 3.13 (m, 1H), 4.23 (m, 1H), 4.52 (m, 1H, partly obscured), 4.55 (s, 2H), 6.75 (br d, 0.5H), 6.98 (d, 1H, J = 8.5), 7.18−7.27 (m, 5H), 7.31 (br d, 1H), 7.67 (br s, 1H), 7.82 (br m, 0.5H). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.7, 28.9, 30.2, 30.4, 38.4, 39.2, 40.7, 56.1, 58.0, 69.2, 81.1, 115.4, 128.0, 129.7, 130.6, 131.7, 133.8, 135.1, 138.8, 157.6, 172.8, 174.3, 174.5. MS (ESI) m/z 597 (M − H). Anal. ($C_{31}H_{42}N_4O_8$·1.5H_2O) C, H, N.

Methyl 2-{4-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-iodophenoxy}acetate (19). Compound **19** was prepared from **3** (2.24 g, 4.70 mmol), according to the general alkylation procedure, which generated 2.29 g (89%) of compound **19** as a white solid. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.88 (t, 3H, J = 7.1, 14.4), 1.20 (m, 2H), 1.29 (m, 2H), 1.38 (m, 2H), 1.43 (s, 9H), 2.96 (m, 2H), 3.17 (m, 2H), 3.81 (s, 3H), 4.19 (m, 1H), 4.67 (s, 2H), 5.06 (br s, 1H), 5.79 (br s, 1H), 6.63 (d, 1H, J = 8.4), 7.12 (dd, 1H, J = 2.1, 8.4), 7.64 (d, 1H, J = 2.1). ^{13}C NMR ($CDCl_3$): δ 14.0, 22.3, 28.3, 28.9, 29.1, 37.2, 39.5, 52.4, 56.0, 66.3, 80.3, 86.6, 112.3, 130.4, 132.3, 140.5, 155.4, 155.7, 168.8, 170.6. MS (ESI) m/z 544 (M − H). Anal. ($C_{22}H_{33}N_2O_6I$) C, H.

Ethyl 3-[5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)phenyl]-2-propynoate (20). Ethyl propiolate (1.94 mL, 19.2 mmol) was added to a suspension of copper(I) oxide (0.61 g, 6.39 mmol) in anhydrous DMF (3 mL) under a nitrogen atmosphere. A solution of **19** (4.38 g, 7,99 mmol) in DMF (40 mL) was added. The reaction flask was flushed with nitrogen, tightly sealed, and stirred at 110 °C for 16 h. The reaction mixture was filtered through a short pad of SiO_2 and washed with EtOAc. The organic layer was washed with 1 M aqueous HCl (20 mL), brine (20 mL), and saturated aqueous $NaHCO_3$ (20 mL), dried (Na_2SO_4), and concentrated. The residue was purified by column chromatography (SiO_4, EtOAc/hexane 1:2 to 1:1), which afforded 1.93 g (47%) of **20** as a white solid; mp 133.9−135.1 °C. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.87 (t, 3H, J = 7.1, 14.4), 1.22 (m, 2H), 1.29 (m, 2H), 1.35 (t, 3H, J = 7.1), 1.41 (s and m, 11H), 2.97 (app t, 2H, J = 6.7, 13.2), 3.17 (m, 2H), 3.80 (s, 3H), 4.21 (m, 1H), 4.29 (q, 2H, J = 7.1), 4.72 (s, 2H), 5.06 (br s, 1H), 5.91 (m, 1H), 6.70 (d, 1H, J = 8.6), 7.22 (dd, 1H, J = 2.1, 8.6), 7.38 (d, 1H, J = 2.1). ^{13}C NMR ($CDCl_3$): δ 13.9, 14.1, 22.2, 28.2, 28.9, 29.1, 37.4, 39.5, 52.3, 55.9, 62.0, 65.9, 80.3, 82.1, 85.0, 109.9, 112.6, 130.3, 133.0, 135.7, 154.0, 155.3, 158.8, 168.7, 170.6. MS (ESI) m/z 517 (M − H). Anal. ($C_{27}H_{38}N_2O_8$·H_2O) C, H, N.

Ethyl 3-[5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)phenyl]-2-propynoate (21). Compound **21** was prepared from **20** (1.01 g, 1.95 mmol) according to the general Boc deprotection and carbodiimide coupling procedure, yielding 1.09 g (84%) of **21** as a white solid; mp 121.8−123.1 °C. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.87 (t, 3H, J = 7.1, 14.4), 1.14−1.41 (m, 6H), 1.34 (t, 3H, J = 7.1), 1.35 (s, 9H), 2.81 (m, 1H), 3.01−3.09 (m, 4H), 3.15 (m, 1H), 3.77 (s, 3H), 4.27 (m, 1H), 4.29 (q, 2H, J = 7.1), 4.56 (m, 1H), 4.69 (s, 2H), 4.97 (d, 1H, J = 6.3), 6.17 (br m, 1H), 6.41 (br m, 1H), 6.66 (d, 1H, J = 8.6), 7.10−7.19 (m, 4H), 7.25−7.34 (m, 3H). ^{13}C NMR ($CDCl_3$): δ 13.9, 14.1, 22.2, 28.1, 28.9, 36.4, 37.7, 39.6, 52.2, 53.6, 56.1, 62.0, 65.8, 80.6, 82.0, 85.0, 109.8, 112.6, 127.2, 128.8, 129.2, 129.9, 133.1, 135.5, 136.0, 153.9, 158.8, 168.6, 169.6, 170.9. MS (ESI) m/z 664 (M − H). Anal. ($C_{36}H_{47}N_3O_9$) C, H, N.

2-[4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(3-hydroxy-5-isoxazolyl)phenoxy]acetic Acid (22). A amount of 2.5 M aqueous NaOH (670 μL, 1.68 mmol) was added to hydroxylamine hydrochloride (61 mg, 0.87 mmol). This mixture was added to a solution of **21** (446 mg, 0.67 mmol) in ethanol/THF (1 mL:2 mL). The clear yellow solution was stirred overnight at ambient temperature and then acidified with 1 M aqueous HCl. The reaction mixture was extracted with EtOAc (2 × 4 mL), and the organic layer was washed with brine (4 mL), dried (Na_2SO_4), and concentrated, which afforded 418 mg of a crude material as a yellowish solid. This material was a mixture of the target compound and the corresponding hydroxamic acid analogues. Separation by reversed phase HPLC furnished 82 mg (19%) of pure title compound **22** as a white solid; mp, sublimed above 260 °C. ^{1}H NMR 500 MHz (MeOH-d_4): δ 0.87 (t, 3H, J = 6.9, 14.4), 1.18 (m, 2H), 1.26 (m, 2H), 1.35 (s, 9H), 1.38 (m, 2H), 2.78 (m, 1H), 3.00−3.07 (m, 4H), 3.16 (dd, 1H), 4.25 (dd, 1H, J = 5.0, 9.1), 4.53 (m, 1H), 4.77 (s, 2H), 6.83 (s, 1H), 7.01 (d, 1H, J = 8.5), 7.16−7.30 (m, 6H), 7.74 (br s, 1H). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.3, 28.6, 29.9, 30.1, 38.2, 38.9, 40.5, 55.9, 57.7, 66.3, 80.9, 97.0, 113.6, 118.1, 127.7, 128.8, 129.4, 130.3, 131.3, 133.4, 138.4, 155.0, 157.8, 166.8, 171.6, 172.5, 172.7, 174.0. HRMS (EI) for $C_{33}H_{42}N_4O_9$: calcd, 638.2952; found, 638.2957. MS (ESI) m/z 637 (M − H). Anal. ($C_{33}H_{42}N_4O_9$ H_2O) C, H, N.

3-[5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(2-hydroxy-2-oxoethoxy)phenyl]-2-propynoic Acid (23). To a solution of **21** (128 mg, 0.19 mmol) in THF (3 mL) was added a 2.5 M aqueous solution of LiOH (309 μL, 0.77 mmol). The mixture was stirred at ambient temperature for 2.5 h at which time TLC showed that still much starting material was left. Additional LiOH (78 μL, 0.19 mmol) was added, and the mixture was stirred for another 2 h. The mixture was acidified

with 3 M aqueous HCl and extracted with EtOAc (5 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford 119 mg (99%) of **23** as a white solid; mp 124.5−130.1 °C. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.90 (t, 3H, J = 7.1, 14.5), 1.19−1.42 (m, 6H), 1.36 (s, 9H), 2.75 (dd, 1H), 2.86 (dd, 1H), 2.90−3.05 (m, 3H), 3.14 (m, 1H), 4.23 (dd, 1H, J = 5.3, 9.2), 4.49 (m, 1H), 4.76 (s, 2H), 6.87 (d, 1H, J = 8.7), 7.18−7.29 (m, 6H), 7.37 (s, 1H). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.4, 28.6, 30.0, 30.1, 30.9, 37.8, 39.0, 40.5, 40.6, 55.7, 57.7, 66.4, 80.9, 83.6, 86.0, 110.6, 113.6, 127.7, 129.4, 130.3, 131.5, 134.3, 136.7, 138.4, 156.8, 160.4, 171.9, 172.4, 174.0. MS (ESI) m/z 622 (M − H). Anal. calcd for $C_{33}H_{41}N_3O_9$: C, 63.55; H, 6.63; N, 6.74. Found: C, 63.0; H, 5.7; N, 6.7.

3-[5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(carboxymethoxy)phenyl]propanoic Acid (24). A mixture of **23** (76 mg, 0.12 mmol) and 10% Pd/C (20 mg) in methanol (4 mL) was hydrogenated at atmospheric pressure for 4 h. The mixture was filtered through Celite, and solvent was removed under reduced pressure to afford 75 mg (98%) of compound **24** as a white solid; mp 99.8−103.2 °C. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.89 (t, 3H, J = 7.1, 14.4), 1.17−1.40 (m, 6H), 1.34 (s, 9H), 2.62 (m, 2H), 2.79 (m, 1H), 2.98 (m, 4H), 3.02 (m, 2H), 3.10 (m, 1H), 4.24 (dd, 1H, J = 5.1, 9.0), 4.49 (br m, 1H), 4.64 (s, 2H), 6.73 (d, 1H, J = 8.2), 6.97 (m, 2H), 7.20−7.28 (m, 5H). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.4, 27.2, 28.6, 29.9, 30.1, 35.1, 38.1, 38.8, 40.5, 55.9, 57.7, 60.2, 66.5, 80.9, 112.5, 127.7, 129.4, 129.4, 130.4, 130.6, 130.7, 132.3, 138.4, 156.4, 157.8, 172.7, 172.8, 173.9, 177.2. MS (ESI) m/z 626 (M − H). Anal. ($C_{33}H_{45}N_3O_9$) C, H, N.

***tert*-Butyl (1*S*)-1-(3-Cyano-4-hydroxybenzyl)-2-oxo-2-(pentylamino)ethylcarbamate (25).** To a solution of **3** (5.94 g, 12.47 mmol) in DMF (anhydrous, 30 mL) in a heavy-walled Pyrex tube fitted with a screw cap was added $Pd(PPh_3)_4$ (0.58 g, 0.50 mmol) and zinc cyanide (1.61 g, 13.72 mmol). The vial was flushed with nitrogen, tightly sealed, and stirred at 80 °C for 16 h. Upon cooling, the mixture was partitioned between EtOAc (50 mL) and 2 M aqueous ammonium hydroxide (50 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by column chromatography (SiO_2, EtOAc/*n*-hexane 1:1) to afford 1.76 g (38%) of compound **25** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.91 (t, 3H, J = 7.3, 14.4), 1.18−1.48 (m, 6H), 1.39 (s, 9H), 2.77 (dd, 1H, J = 8.6, 13.7), 2.98 (dd, 1H, J = 6.45, 13.7), 3.08−3.21 (m, 2H), 4.20 (m, 1H), 6.88 (d, 1H, J = 8.5), 7.34 (dd, 1H, J = 2.2, 8.5), 7.38 (d, 1H, 2.2). ^{13}C NMR (MeOH-d_4): δ 14.7, 23.8, 29.0, 30.4, 30.5, 38.6, 40.8, 57.7, 81.1, 100.8, 117.4, 118.3, 130.6, 135.3, 137.2, 157.9, 160.9, 174.1. MS (ESI) m/z 376 (M + H). Anal. ($C_{20}H_{29}N_3O_4$) C, H, N.

Methyl 2-{4-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-cyanophenoxy}acetate (26). Compound **26** was prepared from **25** (0.61 g, 1.63 mmol) by the general alkylation procedure, which afforded 0.69 g (94%) of compound **26** as a white solid. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.90 (t, 3H, J = 7.1, 14.4), 1.22−1.45 (m, 6H), 1.38 (s, 9H), 2.79 (dd, 1H, J = 8.8, 13.7), 3.01 (m, 1H), 3.11−3.18 (m, 2H), 3.77 (s, 3H), 4.87 (s, 2H), 7.00 (d, 1H, J = 8.7), 7.46 (dd, 1H, J = 2.2, 8.7), 7.51 (d, 1H, J = 2.2). ^{13}C NMR ($CDCl_3$): δ 14.7, 23.7, 28.9, 30.3, 30.4, 38.4, 40.7, 53.0, 57.5, 66.6, 81.0, 103.1, 114.1, 117.4, 132.9, 135.9, 137.1, 157.8, 160.2, 170.4, 173.8. MS (ESI) m/z 448 (M + H). Anal. ($C_{23}H_{33}N_3O_6$) C, H, N.

Methyl 2-{4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-cyanophenoxy}acetate (27). The reaction was carried out with **26** (0.55 g, 1.23 mmol) according to the general Boc deprotection and carbodiimide reaction procedure, yielding 0.61 g (84%) as a white solid; mp 121.4−123.0 °C. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.90 (t, 3H, J = 7.2, 14.5), 1.21 (m, 2H), 1.29 (m, 2H), 1.37 (s, 9H), 1.40 (m, 2H), 2.75 (dd, 1H, J = 9.2, 13.5), 2.91 (dd, 1H, J = 7.7, 13.5), 2.97−3.06 (m, 3H), 3.12 (m, 1H), 3.75 (s, 3H), 4.24 (dd, 1H, J = 5.3, 9.2), 4.51 (app t, 1H, J = 7.1, 14.1), 4.87 (s, 2H), 6.98 (d, 1H, J = 8.6), 7.14−7.27 (m, 5H), 7.44 (dd, 1H, J = 1.9, 8.6), 7.49 (d, 1H, J = 1.9). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.4, 28.6, 30.0, 30.1, 37.7, 39.0, 40.5, 52.7, 55.6, 57.6, 66.4, 80.8, 102.9, 113.9, 117.0, 127.7, 129.4, 130.3, 132.2, 135.6, 136.8, 138.4, 157.7, 160.0, 170.1, 172.2, 174.0. MS (ESI) m/z 593 (M − H). Anal. ($C_{32}H_{42}N_4O_7$) C, H, N.

Methyl 2-[4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(1(2)*H*-tetrazol-5-yl)phenoxy]acetate (28). To a suspension of **27** (0.45 g, 0.75 mmol) in toluene (4 mL), in a heavy-walled Pyrex tube fitted with a screw cap, were added trimethylsilyl azide (0.30 mL, 2.25 mmol) and dibutyltin oxide (19 mg, 0.075 mmol). The mixture was flushed with nitrogen, tightly sealed, and stirred at 110 °C for 16 h. Upon cooling, the volatiles were evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, EtOAc), which afforded 90 mg (19%) of compound **28** as a white solid; mp 189.5−192.8 °C. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.85 (t, 3H, J = 6.9, 14.1), 1.16−1.41 (m, 6H), 1.33 (s, 9H), 2.69 (dd, 1H, J = 9.4, 13.7), 2.95−3.17 (m, 5H), 3.79 (s, 3H), 4.22 (dd, 1H, J = 4.9, 9.4), 4.58 (m, 1H), 4.99 (s, 2H), 7.09−7.24 (m, 6H), 7.42 (d, 1H, J = 1.7, 6.9), 8.09 (d, 1H, J = 1.7). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.4, 28.6, 29.9, 30.1, 38.1, 39.0, 40.5, 53.0, 55.7, 57.7, 66.8, 80.8, 114.0, 114.6, 127.7, 129.4, 130.2, 131.6, 132.7, 135.2, 138.5, 155.6, 157.8, 171.4, 172.4, 174.0. MS (ESI) m/z 636 (M − H). HRMS (EI) calcd for $C_{32}H_{43}N_7O_7$, 637.3224; found, 637.3234. Anal. calcd for $C_{32}H_{43}N_7O_7$: C, 60.27; H, 6.80; N, 15.37. Found: C, 60.7; H, 7.0; N, 14.3.

2-[4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(1(2)*H*-tetrazol-5-yl)phenoxy]acetic Acid (29). To a solution of **28** (19 mg, 0.029 mmol) in THF (0.6 mL) was added a 2.5 M aqueous solution of LiOH (35 μL, 0.087 mmol), and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was then acidified with 3 M aqueous HCl and extracted with EtOAc (2 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford 16 mg (90%) of compound **29** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.85 (t, 3H, J = 7.0, 14.2), 1.18 (m, 2H), 1.24 (m, 2H), 1.33 (s, 9H), 1.38 (m, 2H), 2.70 (dd, 1H, J = 9.4, 13.8), 2.96−3.08 (m, 3H), 3.14 (m, 2H), 4.22 (dd, 1H, J = 5.1, 9.4), 4.58 (m, 1H), 4.95 (s, 2H), 7.12−7.29 (m, 6H), 7.44 (dd, 1H, J = 1.9, 8.5), 7.86 (br m, 0.5H), 8.01 (br m, 1H), 8.10 (d, 1H, J = 1.9). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.4, 28.7, 30.0, 30.2, 38.2, 39.0, 40.6, 55.8, 67.0, 80.9, 114.0, 114.9, 127.8, 129.5, 130.3, 131.5, 132.8, 135.4, 138.5, 155.9, 157.9, 172.5, 173.1, 174.1. MS (ESI) m/z 622 (M − H). HRMS (EI) calcd for $C_{31}H_{41}N_7O_7$, 623.3068; found, 623.3071. Anal. calcd for $C_{31}H_{41}N_7O_7$: C, 59.70; H, 6.63; N, 15.72. Found: C, 59.5; H, 6.7; N, 14.0.

Methyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-(cyanomethoxy)benzoate (30). The reaction was carried out with bromoacetonitrile (103 μL, 1.48 mmol) and compound **5** (0.30 g, 0.74 mmol) according to the general alkylation procedure, which afforded 0.36 g (93%) of compound **30** as a white solid. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.87 (3H, t, J = 7.0, 14.3), 1.18−1.38 (6H, m), 1.41 (9H, s), 3.00 (1H, dd, J = 6.8, 13.8), 3.08 (1H, dd, J = 7.6, 13.8), 3.17 (2H, m), 3.89 (3H, s), 4.26 (1H, m), 4.85 (2H, s), 5.11 (1H, br s), 5.94 (1H, br m), 7.06 (1H, d, J = 8.4), 7.39 (1H, dd, J = 2.2, 8.4), 7.72 (1H, d, J = 2.2). ^{13}C NMR ($CDCl_3$): δ 13.9, 22.3, 28.2, 28.9, 29.0, 37.6, 39.5, 52.3, 55.7, 80.4, 115.0, 116.8, 122.1, 132.6, 133.0, 134.7, 155.1, 156.0, 165.5, 170.6. MS (ESI) m/z 446 (M − H). Anal. ($C_{23}H_{33}N_3O_6$) C, H.

Methyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-(1(2)*H*-tetrazol-5-yl-methoxy)-benzoate (31). To a suspension of **30** (0.21 g, 0.48 mmol) in toluene (1.1 mL), in a heavy-walled Pyrex tube fitted with a screw cap, were added trimethylsilyl azide (127 μL, 0.96 mmol) and dibutyltin oxide (12 mg, 0.05 mmol). The mixture was flushed with nitrogen, tightly sealed, and stirred at 110 °C for 16 h. Upon cooling, the volatiles were evaporated under reduced pressure. The residue was purified by column chromatography (SiO_2, gradient: 5% MeOH in CH_2Cl_2 to 5% MeOH and 1% HOAc in CH_2Cl_2), which afforded 86 mg (37%) of compound **31** as a white solid. ^{1}H NMR 400 MHz (MeOH-

d_4): δ 0.88 (t, 3H, J = 7.0, 14.2), 1.23 (m, 2H,), 1.31 (m, 2H), 1.35 (s, 9H), 1.42 (m, 2H), 2.82 (dd, 1H), 3.05 (dd, 1H), 3.12 (m, 2H), 3.83 (s, 3H), 4.21 (br m, 1H), 5.51 (s, 2H), 7.17 (d, 1H, J = 8.6), 7.42 (dd, 1H, J = 2.2, 8.6), 7.69 (dd, 1H, J = 2.2), 7.93 (br m, 1H). ^{13}C NMR (MeOH-d_4): δ 15.7, 24.7, 29.9, 31.3, 31.4, 39.8, 41.7, 54.0, 58.7, 63.7, 81.9, 117.8, 123.5, 134.1, 134.9, 137.3, 156.5, 158.7, 158.8, 169.3, 175.0. MS (ESI) m/z 491 (M + H). Anal. ($C_{23}H_{34}N_6O_6$) C, H, N.

Methyl 5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(1(2)*H*-tetrazol-5-yl-methoxy)benzoate (32). The reaction was carried out with **31** (40 mg, 0.10 mmol) according to the general Boc deprotection and carbodiimide reaction procedure. The crude product was purified by chromatography (SiO_2, gradient: 5% MeOH in CH_2Cl_2 to 5% MeOH and 1% HOAc in CH_2Cl_2), which gave 21 mg (32%) of compound **32** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.4), 1.20 (m, 2H), 1.28 (m, 2H), 1.35 (s, 9H), 1.37 (m, 2H), 2.75 (dd, 1H), 2.90−3.08 (m, 2H), 3.12 (m, 2H), 3.82 (s, 3H), 4.22 (dd, 1H, J = 5.1, 9.3), 4.50 (br m, 1H), 5.47 (s, 2H), 7.17−7.25 (m, 6H), 7.40 (d, 1H), 7.64 (d, 1H). ^{13}C NMR (MeOH-d_4): δ 13.0, 22.1, 27.3, 28.2, 28.6, 28.8, 36.7, 37.7, 39.2, 51.3, 54.5, 56.5, 61.3, 79.5, 115.1, 121.0, 126.4, 128.0, 128.1, 128.2, 129.0, 129.1, 130.7, 132.3, 134.6, 137.1, 154.8, 156.4, 166.8, 171.1. 172.7. MS (ESI) m/z 636 (M − H). HRMS (EI) calcd for $C_{32}H_{43}N_7O_7$ + Na, 660.3122; found, 660.3100.

5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(1(2)*H*-tetrazol-5-yl-methoxy)benzoic Acid (33). To a solution of **32** (6.7 mg, 0.01 mmol) in THF (0.2 mL) was added a 2.5 M aqueous solution of LiOH (13 μL, 0.03 mmol), and the mixture was stirred at ambient temperature for 2 h. The reaction mixture was then acidified with 3 M aqueous HCl and extracted with EtOAc (2 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford 6 mg (99%) of compound **33** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.88 (t, 3H, J = 7.1, 14.4), 1.2−1−4 (m, 6H), 1.35 (s, 9H), 2.78 (m, 1H), 2.95−3.18 (m, 5H), 4.22 (dd, 1H), 4.52 (br m, 1H), 5.53 (s, 2H), 7.16−7.27 (m, 6H), 7.42 (dd, 1H), 7.72 (d, 1H). ^{13}C NMR (MeOH-d_4): δ 14.6, 23.6, 28.9, 29.8, 30.2, 30.4, 31.2, 38.3, 39.3, 40.8, 56.1, 58.0, 62.8, 81.2, 116.7, 123.0, 128.0, 128.2, 129.6, 129.7, 129.8, 130.6, 130.7, 132.7, 134.3, 136.2, 138.7, 157.9, 169.6, 172.7, 174.3. MS (ESI) m/z 622 (M − H). HRMS (EI) calcd for $C_{31}H_{41}N_7O_7$ + Na, 646.2965; found, 646.2951.

(*S*)-*N*-[2-(4-Carboxymethoxy-3-cyano-phenyl)-1-pentylcarbamoyl-ethyl]succinamic Acid (34). Compound **34** was prepared from **26** according to the general method described for the Boc deprotection and carbodiimide coupling using succinic anhydrid. Hydrolysis of the methylester using LiOH in THF afforded the target compound **34** in 82% yield as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.92 (t, 3H, J = 7.0, 14.3 Hz), 1.24 (m, 2H), 1.33 (m, 2H), 1.45 (m, 2H), 2.43−2.61 (m, 4H), 2.87 (dd, 1H, J = 8.5, 13.9 Hz), 3.08−3.17 (m, 3H), 4.53 (dd, 1H, J = 6.3, 8.5), 4.85 (s, 2H), 7.00 (d, 1H, J = 8.7 Hz), 7.49 (dd, 1H, J = 2.1, 8.7 Hz), 7.53 (1H, d, J = 2.1 Hz). ^{13}C NMR (MeOH-d_4): δ 14.7, 23.8, 30.4, 30.4, 30.5, 31.7, 37.8, 40.9, 56.4, 66.6, 103.2, 114.2, 117.5, 132.7, 135.8, 137.0, 160.5, 171.8, 173.2, 175.0, 176.7. MS (ESI) m/z 432 (M − H). Anal. calcd for $C_{21}H_{27}N_3O_7 \cdot 1/2H_2O$: C, 57.01; H, 6.38; N, 9.49. Found: C, 57.0; H, 6.4; N, 8.7.

5-[(2*S*)-2-(3-Carboxy-propionylamino)-2-pentylcarbamoyl-ethyl]-2-cyanomethoxy-benzoic Acid (35). Compound **35** was prepared from **30** according to the general method described for the Boc deprotection and carbodiimide coupling using succinic anhydrid. Hydrolysis of the methylester using LiOH in THF afforded the target compound **35** in 82% yield as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.90 (t, 3H, J = 7.2, 14.5 Hz), 1.22 (m, 2H), 1.31 (m, 2H), 1.42 (m, 2H), 2.40−2.60 (m, 4H), 2.89 (dd, 1H, J = 8.5, 13.8 Hz), 3.08−3.19 (m, 3H), 4.52 (m, 1H), 4.60 (s, 2H), 7.05 (d, 1H, J = 8.5 Hz), 7.46 (dd, 1H, J = 2.3, 8.5 Hz), 7.83 (d, 1H, J = 2.3 Hz). ^{13}C NMR (MeOH-d_4): δ 14.7, 23.8, 30.3, 30.5, 30.5, 31.8, 38.3, 40.8, 56.6, 69.2, 115.7, 121.5, 132.3, 134.5, 136.5, 158.4, 169.3, 173.4, 174.4, 174.9, 176.7. MS (ESI) m/z 446 (M − H). Anal. ($C_{22}H_{29}N_3O_7 \cdot 1.5H_2O$) C, H, N.

[2-(5-Oxo-2,3-dihydro-5H-benzo[*e*][1,4]dioxepin-7-yl)-(1*S*)-1-pentylcarbamoyl-ethyl]carbamic Acid *tert*-Butyl Ester (36). To a solution of **4** (359 mg, 0.74 mmol) (can also use **5**) were added 2-bromoethanol (157.8 μL, 2.23 mmol) and K_2CO_3 in DMF (3 mL) in a Heck vial. The vial was tightly sealed, and the mixture was stirred at 80 °C overnight. The reaction mixture was cooled and diluted with EtOAc (5 mL). The organic layer was washed with 10% aqueous HCl, dried (Na_2SO_4), and concentrated. Purification was by flash chromatography (SiO_2, EtOAc/*n*-hexane 1:1), which furnished 65 mg (21%) of **36** as a white solid. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.87 (t, 3H), 1.23 (m, 2H), 1.28 (m, 2H), 1.40 (s, 9H), 1.41 (partly obscured by singlett, m, 2H), 2.97 (dd, 1H), 3.09 (dd, 1H), 3.19 (m, 2H), 4.28 (m, 1H), 4.47 and 4.47 (two m, 2H + 2H, coupling according to COSY), 5.12 (broad m, 1H), 6.06 (broad t, 1H), 6.94 (d, 1H, J = 8.4 Hz), 7.36 (dd, 1H, J = 2.1, 8.4 Hz), 7.71 (d, 1H, J = 2.1 Hz). ^{13}C NMR ($CDCl_3$): δ 13.9, 22.3, 28.9, 29.1, 37.5, 39.5, 55.7, 65.6, 70.8, 118.8, 121.3, 131.4, 134.1, 136.1, 153.7, 155.4, 169.0, 170.7. MS (ESI) m/z 421 (M + H). Anal. ($C_{22}H_{32}N_2O_6$) C, H, N.

***N*-[2-(5-Oxo-2,3-dihydro-5H-benzo[*e*][1,4]dioxepin-7-yl)-(1*S*)-1-pentylcarbamoyl-ethyl]succinamic Acid (37).** Compound **37** was prepared from **36** (43 mg, 0.13 mmol) according to the general method described for the Boc deprotection and carbodiimide coupling using succinic anhydride. Purification of the crude product by flash chromatograpy (SiO_2, gradient: MeOH/CH_2Cl_2 5:95 to HOAc/MeOH/CH_2Cl_2 1:5:94) afforded 26 mg (42%) of **37** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.91 (t, 3H), 1.25 (m, 2H), 1.32 (m, 2H), 1.45 (m, 2H), 2.4−2.6 (2m, 2H+2H), 2.88 (dd, 1H, J = 8.8, 13.8 Hz), 3.1−3.22 (m, 3H), 4.50 (s, 4H), 4.53 (m, 1H), 6.99 (d, 1H, J = 8.4 Hz), 7.45 (dd, 1H, J = 2.3, 8.4 Hz), 7.70 (d, 1H, J = 2.3 Hz), 7.96 (broad m, 1H). ^{13}C NMR (MeOH-d_4): δ 16.6, 25.6, 32.2, 32.3, 32.4, 33.6, 40.1, 42.7, 58.3, 69.3, 74.5, 122.8, 124.6, 135.4, 137.0, 139.4, 157.5, 173.8, 175.2, 176.8, 178.6. MS (ESI) m/z 421 (M + H). Anal. ($C_{21}H_{28}N_2O_7$) C, H, N.

5-[(1*S*)-2-(3-Carboxy-propionylamino)-2-pentylcarbamoyl-ethyl]-2-(2-hydroxyethoxy)benzoic Acid (38). To a solution of **37** (20 mg, 0.048 mmol) in THF (0.6 mL) was added a 2.5 M aqueous solution of LiOH (38 μL, 0.095 mmol). The mixture was stirred at ambient temperature for 2.5 h. The mixture was diluted with EtOAc (3 mL) and extracted with saturated aqueous $NaHCO_3$ (2 × 3 mL). The water layer was acidified with 3 M aqueous HCl and extracted with EtOAc (2 × 2 mL). The organic layer was dried (Na_2SO_4) and concentrated to afford 16 mg (75%) of **38** as a white solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.91 (t, 3H), 1.23 (m, 2H), 1.31 (m, 2H), 1.43 (m, 2H), 2.4−2.62 (2m, 2H+2H), 2.88 (dd, 1H), 3.05−3.2 (m, 3H), 3.89 (m, 2H), 4.21 (m, 2H), 4.53 (m, 1H), 7.10 (d, 1H, J = 8.5 Hz), 7.42 (dd, 1H, J = 2.3, 8.5 Hz), 7.76 (d, 1H, J = 2.3 Hz), 7.93 (broad m, 1H). ^{13}C NMR (MeOH-d_4): δ 16.8, 25.8, 31.9, 32.2, 32.4, 32.5, 33.8, 40.3, 42.9, 58.7, 63.3, 74.9, 117.8, 123.5, 133.8, 136.2, 138.4, 161.2, 171.9, 175.5, 176.9, 178.7. MS (ESI) m/z 439 (M + H). Anal. ($C_{21}H_{30}N_2O_8$) C, H, N.

[(1*S*)-2-(3-Acetyl-4-hydroxy-phenyl)-1-pentylcarbamoyl-ethyl]carbamic Acid *tert*-Butyl Ester (39). In an oven-dried, 25 mL, heavy-walled, and thin-necked Pyrex tube fitted with a screw cap were combined **3** (440 mg, 0.92 mmol), palladium(II) acetate (6.2 mg, 3 mol %), 1,3-bis(diphenylphosphino)propane (DPPP, 23 mg, 6 mol %), and thallium(I) acetate (263 mg, 0.998 mmol). The mixture was suspended in DMF (2 mL), and butyl vinyl ether (0.26 mL, 1.85 mmol) was added. The flask was purged with nitrogen and tightly sealed, and the reaction mixture was stirred 90 °C for 16 h. The mixture was to cooled to room temperature, diluted with THF (2 mL), and treated with 10% aqueous HCl (2 mL). After it was stirred for 30 min, the mixture was extracted with EtOAc (2 × 2 mL) and washed with brine (3 × 2 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc/*n*-hexane 1:2), which furnished 150 mg (41%) of **39** as a colorless oil. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.86 (t, 3H, J = 7.1, 14.4), 1.16 (m, 2H), 1.26 (m,

2H), 1.40 (m, 2H), 1.42 (s, 9H), 2.62 (s, 3H), 3.01 (m, 2H), 3.16 (m, 2H), 4.25 (m, 1H), 5.05 (br s, 1H), 5.81 (br m, 1H), 6.91 (d, 1H, $J = 8.5$), 7.32 (dd, 1H, $J = 8.5, 2.0$), 7.56 (d, 1H, $J = 2.0$). ^{13}C NMR ($CDCl_3$): δ 13.9, 22.3, 26.7, 28.3, 28.9, 29.1, 37.7, 39.5, 56.0, 80.0, 118.6, 119.6, 127.2, 131.3, 131.3, 137.5, 161.3, 170.7, 204.4. Anal. ($C_{21}H_{32}O_5N_2$) C, H.

[(1*S*)-2-Acetyl-4-(2-*tert*-butoxycarbonylamino-2-pentylcarbamoyl-ethyl)phenoxy]acetic Acid Methyl Ester (40). A mixture of **39** (294 mg, 0.75 mmol), methyl bromoacetate (142 μL, 1.50 mmol), and freshly ground K_2CO_3 (207 mg, 1.50 mmol) was suspended in acetone (4 mL). The mixture was stirred at room temperature for 48 h, after which time H_2O (2 mL) was added and the mixture was extracted with EtOAc (3 mL). The organic layer was washed with brine (2 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc/*n*-hexane 1:1), which furnished 126 mg (36%) of **40** as a white solid. ^{1}H NMR 400 MHz ($CDCl_3$): δ 0.87 (t, 3H, $J = 7.1, 14.3$), 1.21 (m, 2H), 1.28 (m, 2H), 1.39 (m, 2H), 1.40 (s, 9H), 2.69 (s, 3H), 2.98 (m, 1H), 3.04 (m, 1H), 3.17 (m, 2H), 3.81 (s, 3H), 4.23 (m, 1H), 4.71 (s, 2H), 5.03 (br s, 1H), 5.86 (br m, 1H), 6.75 (d, 1H, $J = 8.6$), 7.31 (dd, 1H, $J = 2.3, 8.6$), 7.59 (d, 1H, $J = 2.3$). ^{13}C NMR ($CDCl_3$): δ 13.9, 22.3, 28.2, 28.9, 29.1, 32.0, 37.4, 39.5, 52.4, 55.8, 65.4, 80.3, 112.5, 128.6, 130.3, 131.4, 134.4, 155.9, 168.6, 170.7, 199.3. Anal. ($C_{24}H_{36}O_7N_2$) C, H.

***N*-[(1*S*)-2-(3-Acetyl-4-methoxycarbonylmethoxy-phenyl)-1-pentylcarbamoyl-ethyl]succinamic Acid (41).** Trifluoroacetic acid (0.16 mL, 2.1 mmol) was carefully added to a stirring solution of **40** (116 mg, 0.25 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred for 3 h allowing the solution to warm to ambient temperature. The volatiles were removed in vacuo, and the residue was partitioned between EtOAc (3 mL) and saturated aqueous $NaHCO_3$ (3 mL). The organic layer was dried ($MgSO_4$) and concentrated to dryness to afford 94 mg (>100%) of the crude amine as a colorless oil. The amine was dissolved in CH_2Cl_2 (3 mL) and cooled with ice to 0 °C. Succinic anhydride (25 mg, 0.25 mmol) and triethylamine (77 μL, 0.55 mmol) were added, and the mixture was stirred for 16 h allowing the solution to warm to ambient temperature. The mixture was diluted with CH_2Cl_2 (5 mL), and the organic phase was washed with 10% aqueous HCl (2 × 3 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (SiO_2, mobile impurities were eluted with 5% MeOH in CH_2Cl_2 and then 5% MeOH/1% HOAc in CH_2Cl_2 to bring the product). The collected fractions were concentrated, and the remaining HOAc was removed by azeotroping with toluene on a rotavapor and then drying overnight under high vacuum, which furnished 75 mg (64%) of **41** as a white/yellow solid. ^{1}H NMR 400 MHz (MeOH-d_4): δ 0.91 (t, 3H, $J = 7.1, 14.4$), 1.23 (m, 2H), 1.31 (m, 2H), 1.43 (m, 2H), 2.38−2.59 (m, 4H), 2.68 (s, 3H), 2.86 (dd, 1H, $J = 8.7, 13.8$), 3.09−3.17 (m, 3H), 3.80 (s, 3H), 4.51 (dd, 1H, $J = 6.1, 8.7$), 4.87 (s, 2H), 6.97 (d, 1H, $J = 8.6$), 7.40 (dd, 1H, $J = 2.4, 8.6$), 7.59 (d, 1H, $J = 2.4$), 7.93 (br t, 1H), 8.25 (br d, 1H). ^{13}C NMR (MeOH-d_4): δ 14.7, 23.8, 30.3, 30.5, 30.6, 31.8, 32.5, 38.2, 41.0, 53.0, 56.6, 66.6, 114.4, 129.8, 132.2, 132.4, 136.1, 158.0, 170.9, 173.5, 175.0, 176.8, 202.3. MS (ESI) 463 (M − H). Anal. ($C_{23}H_{32}O_8N_2$) C, H.

***N*-[(1*S*)-2-(3-Acetyl-4-carboxymethoxy-phenyl)-1-pentylcarbamoyl-ethyl]succinamic Acid (42).** A solution of **41** (65 mg, 0.14 mmol) and 2.5 M aqueous LiOH (223 μL, 0.56 mmol) in THF/MeOH/H_2O 3:1:1 (3 mL) was stirred at ambient temperature for 16 h. The reaction mixture was acidified with 10% aqueous HCl and extracted with EtOAc (4 × 2 mL). The organic layer was dried ($MgSO_4$) and concentrated to dryness, which furnished 37 mg (59%) of **42**. ^{1}H NMR 500 MHz (MeOH-d_4): δ 0.88 (t, 3H, $J = 7.2, 14.5$), 1.22 (m, 2H), 1.29 (m, 2H), 1.40 (m, 2H), 2.38−2.58 (m, 4H), 2.66 (s, 3H), 2.84 (dd, 1H, $J = 8.5, 13.9$), 3.08−3.17 (m, 3H), 4.49 (dd, 1H, $J = 6.3, 8.5$), 4.77 (s, 2H), 6.95 (d, 1H, $J = 8.5$), 7.37 (dd, 1H, $J = 2.3, 8.5$), 7.56 (d, 1H, $J = 2.3$). ^{13}C NMR (MeOH-d_4): δ 14.3, 23.4, 29.9, 30.11, 30.14, 31.4, 32.1, 37.9, 40.5, 56.2, 66.3, 114.1, 129.4, 131.6, 132.0, 135.7, 157.8, 171.9, 173.1, 174.5, 176.3, 202.1. MS (ESI) 449 (M − H). Anal. ($C_{22}H_{30}O_8N_2$) C, H.

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Supporting Information Available: Combustion analyses for new compounds. Crystallography materials and methods for PTP1B−compound **29** complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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(30) Caco-2 cell results: **29** Papp a→b 1.9 ± 1.4 × 10^{-7} cm/s, Papp b→a 5.1 ± 0.3 × 10^{-7} cm/s. **2** Papp a→b <1 × 10^{-7} cm/s, Papp b→a <1 × 10^{-7} cm/s. **11** Papp a→b 1.3 ± 1.4 × 10^{-7} cm/s, Papp b→a −. **22** Papp a→b <0.8 × 10^{-7} cm/s, Papp b→a <0.5 × 10^{-7} cm/s. Concentration, 100 μM; *b* = basolateral (serosal) side; *a* = apical (mucosal) side. Caco-2 permeability experiments: the compound is dissolved in a physiological buffer and added to one side of a confluent cell monolayer. The rate of appearance of compound on the opposite side of the monolayer is measured. Permeability across the monolayer is calculated according to $P_{app} = dQ/(dt \cdot Co \cdot A)$ where dQ/dt is the permeability rate (steady state flux, mol/s), Co is the initial concentration in the donor chamber (mol/mL), and *A* is the surface area of the monolayer (cm^2). P_{app} (apparent permeability) is expressed as cm/s. A compound with a high (Caco-2) permeability will most likely penetrate the cell membrane and reach into the cell, while low permeability drugs have difficulties getting into the cells and thus difficulties reaching an intracellular target enzyme. It is established that Caco-2 cells express efflux proteins. Therefore, experiments are performed in both mucosal to serosal and serosal to mucosal directions in order to reveal "false" low permeability values.

(31) Data are rates of uptake of 2-deoxyglucose by L6 myocytes in the absence and presence of insulin at a concentration of 10 nM, which supports half-maximal uptake. Two measurements were performed with a concentration of compoound **29** of 100 μM. Data are expressed as percent basal (unstimulated) in the absence of compound and as percent insulin-stimulated, i.e., [(uptake with insulin plus test cpd) − (uptake without insulin plus test cpd)]/[(uptake with insulin no test cpd) − (uptake without insulin no test cpd)] × 100%.

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