Synthesis and Biological Activity of a Novel Class of Small Molecular Weight Peptidomimetic Competitive Inhibitors of Protein Tyrosine Phosphatase 1B

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Protein tyrosine phosphatase 1B (PTP1B) negatively regulates insulin signaling in part by dephosphorylating key tyrosine residues within the regulatory domain of the β-subunit of the insulin receptor (IR), thereby attenuating receptor tyrosine kinase activity. Inhibition of PTP1B is therefore anticipated to improve insulin resistance and has recently become the focus of discovery efforts aimed at identifying new drugs to treat type II diabetes. We previously reported that the tripeptide Ac-Asp-Tyr(SO_3H)-Nle-NH_2 is a surprisingly effective inhibitor of PTP1B (K_i = 5 μM). With the goal of improving the stability and potency of this lead, as well as attenuating its peptidic character, an analogue program was undertaken. Specific elements of the initial phase of this program included replacement of the N- and C-termini with non-amino acid components, modification of the tyrosine subunit, and replacement of the tyrosine sulfate with other potential phosphate mimics. The most potent analogue arising from this effort was triacid **71**, which inhibits PTP1B competitively with a K_i = 0.22 μM without inhibiting SHP-2 or LAR at concentrations up to 100 μM. Overall, the inhibitors generated in this work showed little or no enhancement of insulin signaling in cellular assays. However, potential prodrug triester **70** did induce enhancements in 2-deoxyglucose uptake into two different cell lines with concomitant augmentation of the tyrosine phosphorylation levels of insulin-signaling molecules. Key elements of the overall SAR reported herein include confirmation of the effectiveness and remarkable PTP1B-specificity of the novel tyrosine phosphate bioisostere, *O*-carboxymethyl salicylic acid; demonstration that the tyrosine skeleton is optimal relative to closely related structures; replacement of the p-1 aspartic acid with phenylalanine with little effect on activity; and demonstration that inhibitory activity can be maintained in the absence of an N-terminal carboxylic acid. An X-ray cocrystal structure of an analogue bearing a neutral N-terminus (**69**) bound to PTP1B is reported that confirms a mode of binding similar to that of peptidic substrates.

Introduction

An important component of the current therapy for type II diabetes has been the administration of agents that augment secretion of insulin from the pancreas. While this effectively reduces circulating plasma glucose levels in some patients, it does not address the underlying factor responsible for the hyperglycemia, namely the insulin resistance of the peripheral tissues.[1] Under normal physiological conditions, insulin exerts its effects via initially binding to the extracellular domain (α-subunit) of the insulin receptor (IR), thereby effecting a conformational change in the intracellular domain (β-subunit) of the IR. This in turn activates an intrinsic tyrosine kinase present on the β-subunit, resulting in the phosphorylation of key tyrosine residues within the β-subunit (autophosphorylation).[2a] In this phosphorylated state, the IR possesses high tyrosine kinase activity toward proximal signaling molecules such as insulin receptor substrate-1 (IRS-1). Following multiple tyrosyl phosphorylations, IRS-1 propagates the insulin signal by docking with and subsequently activating a number of effector proteins. The insulin signal branches at this point, eventually leading to the multiple observed responses to insulin that occur within minutes (e.g., glucose uptake) or many hours (e.g., mitogenesis).[2b] The underlying defect in type II diabetes is an attenuation of this insulin-signaling cascade, apparently originating at or near the level of the IR itself.[3]

One strategy to combat this insulin resistance therapeutically may be to maintain insulin receptors (IR) in the active tyrosine-phosphorylated form by inhibiting enzymes that catalyze IR dephosphorylation. There is evidence that protein tyrosine phosphatase 1B (PTP1B) catalyzes IR dephosphorylation and is involved physiologically and pathologically in terminating insulin signaling. This evidence includes descriptions of both in vitro[4,5] and in vivo[6] interactions between PTP1B and IR. Purified PTP1B dephosphorylates IR, apparently in the sequence observed in vivo, and dephosphorylation results in loss of IR tyrosine kinase activity.[7] Intracellular delivery of neutralizing antibodies against PTP1B was found to augment IR and IRS-1 phosphorylation,[8]

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while conversely, overexpression of PTP1B in transfected cells inhibited IR and IRS-1 phosphorylation.[9] Furthermore, PTP1B activity is increased in some tissues of obese insulin-resistant patients[10] and is reduced in parallel with the increased insulin sensitivity that accompanies weight loss.[10,11] Experimentally induced insulin resistance in a variety of cell models is accompanied by an increase in PTP1B activity.[12,13] In addition, correction of glucose-induced insulin resistance in Rat1 fibroblasts with a thiazolidinedione insulin sensitizer also corrects the elevation of PTP1B activity in the insulin-resistant cells.[13] Recently, it was reported that PTP1B null mice have increased insulin sensitivity and decreased susceptibility to diet-induced obesity, but otherwise appear normal.[14]

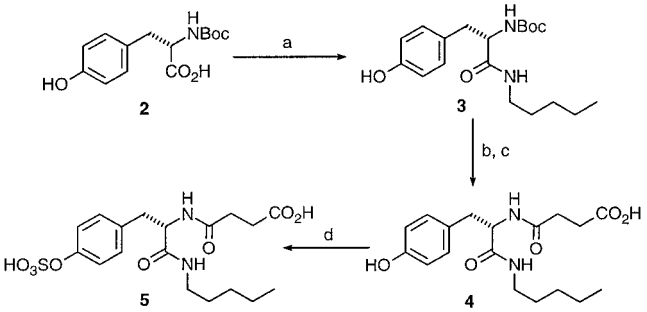
Inhibition of PTP1B may thus be an effective means by which to treat type II diabetes, and research in this field has accelerated dramatically in recent years. Because the catalytic activity of PTP1B relies on the viability of an active site cysteine thiol (Cys215), inhibition of the enzyme can be readily achieved by simple complexation with metals, chemical oxidation, or capture with electrophiles.[15] It is unlikely, however, that any of these approaches will provide inhibitors with specificity for PTP1B over the many other PTP enzymes present in cells, a requirement that would be expected for a safe drug with minimal side effects. An alternative approach, which is more likely to be selective, is to develop reversible competitive inhibitors that mimic the natural substrate, a phosphotyrosine-containing polypeptide. Because tyrosine phosphates are chemically labile, intensive research has been undertaken to develop chemically and enzymatically stable mimics that maintain high affinity for the enzyme active site.[16,17] Examples of functional groups successfully employed as phosphate mimics in PTP1B inhibitors include methylenephosphonate, difluoromethylenephosphonate, sulfate, malonate, and carboxylate.[17]

At the outset of the present work, it was generally accepted that a minimum of six amino acid residues was required for peptidic substrates or inhibitors to possess high affinity for PTP1B.[18] We were, therefore, surprised to discover that the simple tripeptide Ac-NH-Asp-Tyr-(SO_3H)-Nle-NH_2 (**1**) is a highly effective inhibitor of PTP1B (K_i = 5 μM).[19] A similar result was recently obtained by Ramachandran et al., who reported that the simple sulfotyrosyl-containing tetrapeptide AcDE(sY)-Nle inhibits PTP1B with an IC_{50} of 2 μM.[20] Because of the relatively low molecular weight of our tripeptide inhibitor, we were encouraged to embark on an analogue program aimed at developing peptidomimetic inhibitors based on this peptide lead, an endeavor that ultimately led to the identification of submicromolar inhibitors of relatively low molecular weight (<600 Da) and the achievement of insulin-sensitizing effects in cells.[19] In this report, we provide details of the design, synthesis, and structure−activity relationships of this novel class of PTP1B inhibitors.

Chemistry

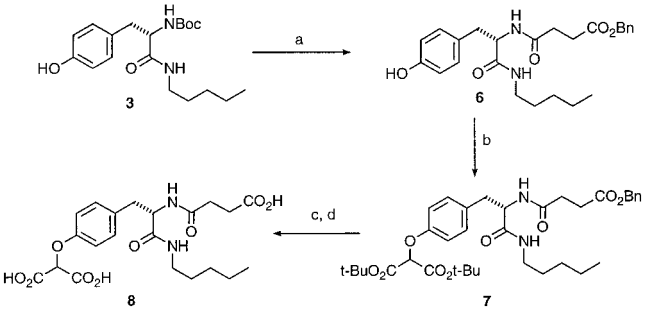
A simplified version of tripeptide **1** lacking the terminal amides was prepared as in Scheme 1. Amidation of *N*-Boc-L-tyrosine **2** with *n*-pentylamine followed by amine deprotection and acylation with succinic anhydride afforded succinamide **4**. Sulfation of the phenolic oxygen with sulfur trioxide−pyridine then generated sulfotyrosine analogue **5**.

Scheme 1



Reagents: (a) *n*-$C_5H_{11}NH_2$, EDC; (b) HCl/dioxane; (c) succinic anhydride, TEA, CH_2Cl_2; (d) SO_3−pyridine, DMF.

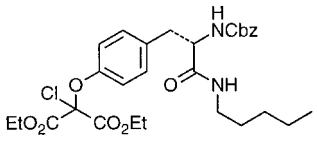
Scheme 2



Reagents: (a) (i) HCl/dioxane, (ii) benzyl succinate, EDC, TEA; (b) di-*tert*-butyl diazomalonate, $Rh_2(OAc)_4$, benzene; (c) TFA, CH_2Cl_2; (d) H_2, Pd/C, MeOH.

Preparation of an *O*-malonate analogue of **5** is described in Scheme 2. Deprotection of **3** and acylation with monobenzyl succinate provided benzyl ester **6**. Rhodium-catalyzed alkylation of the phenol with di-*tert*-butyl diazomalonate[21] afforded triester **7**, which was sequentially deprotected with TFA and catalytic hydrogenation to provide the desired triacid **8**. Basic hydrolysis of the esters was avoided at this point to minimize the chances of racemization at the chiral center.

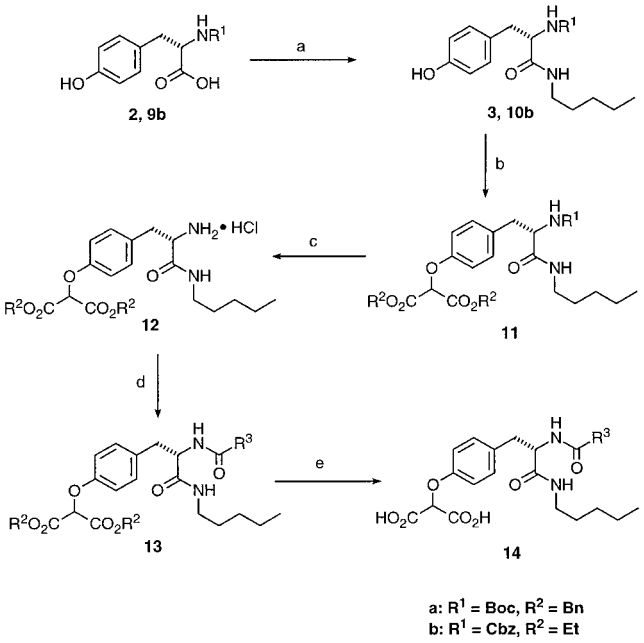
The route in Scheme 2 had to be modified to allow facile generation of analogues with varying substituents at the N- and C-termini. Two different routes to N-terminal analogues are depicted in Scheme 3. Alkylation of protected tyrosines **3** and **10** with 2-halomalonate esters afforded the *O*-malonates **11**. We consistently observed the generation of small amounts of halogenated byproducts in these alkylations (e.g., **15**) that could not be easily removed chromatographically.[22]



15

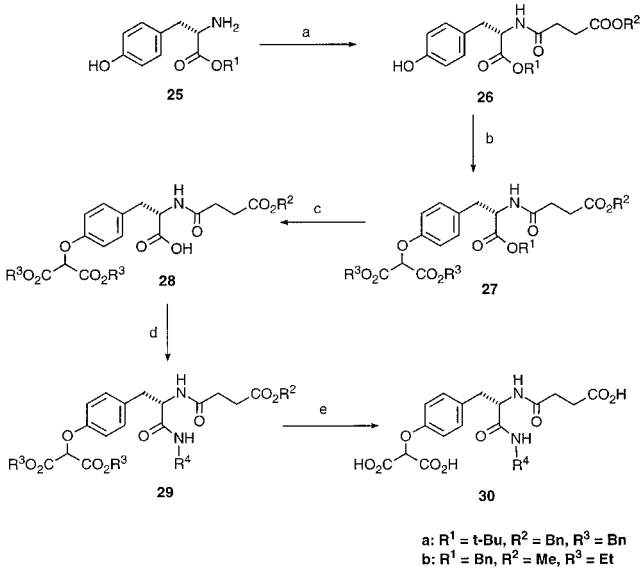
Fortunately, subsequent catalytic hydrogenations effectively converted these side products into the desired products, presumably via facile reduction of the halogens. Urethanes **11** were deprotected with either HCl or catalytic hydrogenation to afford amines **12**. Acylation of the free amines could be effected either directly

Scheme 3



Reagents: (a) *n*-$C_5H_{11}NH_2$, EDC; (b) dibenzyl bromomalonate or diethyl chloromalonate, K_2CO_3, acetone; (c) HCl/HOAc or H_2, Pd/C; (d) R^3CO_2H, EDC or $(R^3CO)_2O$, TEA; (e) H_2, Pd/C or LiOH, THF/MeOH.

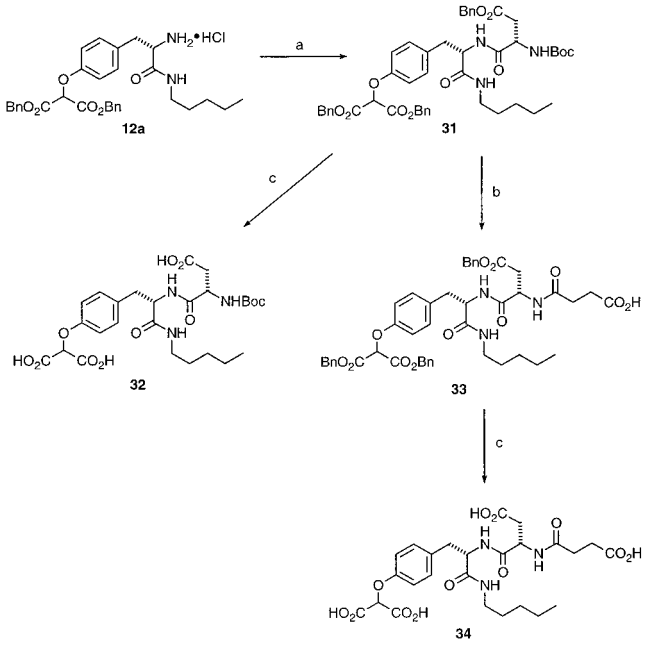
Scheme 4



Reagents: (a) benzyl or methyl succinate, EDC, CH_2Cl_2; (b) dienzyl bromomalonate or diethyl chloromalonate, K_2CO_3, acetone; (c) TFA/CH_2Cl_2 or H_2, Pd/C; (d) R^4NH_2, EDC, CH_2Cl_2; (e) H_2, Pd/C or LiOH, THF/MeOH.

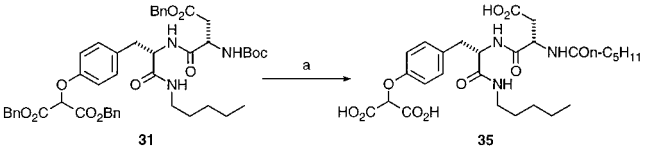
with various anhydrides or with carboxylic acids under the influence of EDC to give amides **13**. Final removal of the malonate esters was then accomplished either by catalytic hydrogenation or saponification with LiOH, affording malonic acids **14** (specific analogues are compiled in Table 2). The latter conditions have been frequently reported to successfully effect ester hydrolyses without racemization of amino acid chiral centers. We confirmed this in our own work with later diastereomeric analogues (vide infra). Synthetic routes analogous to that depicted for the conversion of **2** to **14** in Scheme 3 were used to prepare analogues incorporating

Scheme 5



Reagents: (a) Boc-Asp(OBn)OSu, TEA; (b) (i) HCl/HOAc, ii. succinic anhydride, TEA; (c) H_2, Pd/C.

Scheme 6



Reagents: (a) (i) HCl/HOAc, (ii) *n*-$C_5H_{11}COCl$, (iii) H_2, Pd/C.

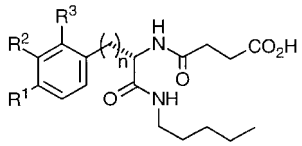
a modified or substituted tyrosine core. Thus, starting with racemic *N*-Boc-homotyrosine, *o*-fluorotyrosine, and pentafluorotyrosine, analogues **19**, **20**, and **21** (Table 1) were obtained, respectively.

Scheme 4 similarly outlines two alternate routes to analogues modified at the C-terminus. Tyrosine ester **25** was acylated with monosuccinate esters as previously described. Alkylation with halomalonate esters proceeded as before to provide the desired malonate ethers **27** in good yields. Selective deprotection of the C-terminal ester was effected with either TFA or via catalytic hydrogenation, and the free acid could be converted to various amides **29** with EDC and the appropriate amine. Final deprotection via hydrogenation or saponification afforded the desired triacids **30**, specific examples of which are compiled in Table 2.

Insertion of an aspartic acid residue is outlined in Scheme 5. Coupling of amine **12a** with *N*-Boc-aspartic acid benzyl ester provided bis(amide) **31**, which could be directly deprotected to triacid **32** via hydrogenation. Alternatively, the Boc group could be removed and the reslulting free amine could be acylated with succinic anhydride to afford mono acid **33**. Hydrogenolytic removal of the benzyl esters then afforded tetraacid **34**. The N-terminal amine could be capped with other acyl groups, as exemplified in Scheme 6.

A route analogous to Scheme 5 was employed to insert phenylalanine-derived amino acids, although here the malonate was protected as the corresponding diethyl ester (exemplified in Scheme 7). After amide coupling

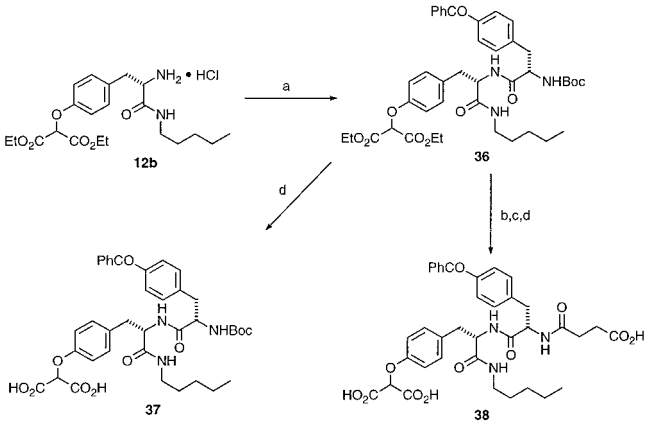
Table 1. Modifications of the Tyrosine Nucleus and Phosphate Bioisosteres



cmpd	R^1	R^2	R^3	n	% inhibition of phosphatases[a] (K_i) PTP1	PTP1B	LAR	SHP-2
5	OSO_2OH	H	H	1	80 (30 μM)	51	5	6
8	$OCH(CO_2H)_2$	H	H	1	76 (12 μM)	80 (11 μM)	2	8
19[b]	$OCH(CO_2H)_2$	H	H	2	18		0	10
20[b]	$OCH(CO_2H)_2$	F	H	1	78		4	9
21[c]	$OCH(CO_2H)_2$	F	F[d]	1	85		12	23
40	$OCOCH_2CO_2H$	H	H	1	2			0
41	OCH_2CO_2H	H	H	1	14		0	0
42	OSO_2CH_3	H	H	1	0			
45	$OC(CO_2H)$═$C HCO_2H$	H	H	1	50			5
46	$OCH(CO_2H)CH_2CO_2H$	H	H	1	34			4
50	CH═$C(CO_2H)_2$	H	H	1	33		2	52
51	$CH_2CH(CO_2H)_2$	H	H	1	34		3	3
57	OCH_2CO_2H	CO_2H	H	1	93 (2.8 μM)	87 (6.5 μM)	0	5
59	OH	CO_2H	H	1	0		0	3
63	OCH_2CONH_2	CO_2H	H	1	16	5	0	2
67	OCH_2CO_2H	$CONH_2$	H	1	11		0	1
72 (*R*)	$OCH(CO_2H)_2$	H	H	1	39		1	4
73	$OCH(CO_2Et)_2$	H	H	1	1			0
74	OSO_2CF_3	H	H	1	0			

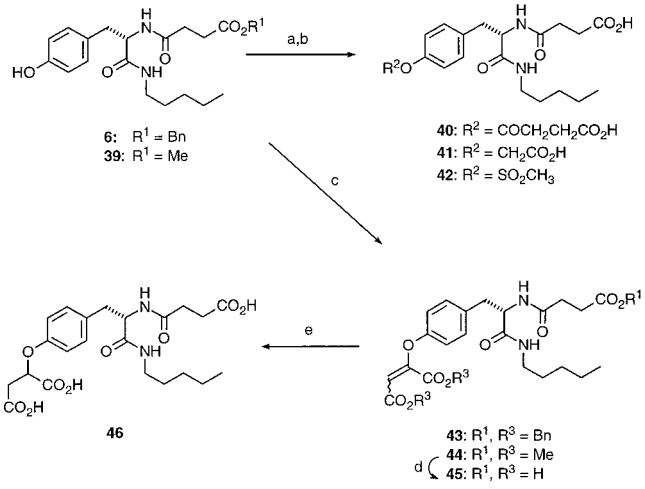
[a] Assayed at 100 μM. [b] Racemate. [c] Most active enantiomer (undetermined). [d] Tyrosine ring perfluorinated

Scheme 7



Reagents: () L-Boc-Bpa-OH, EDC, TEA; (b) HCl/HOAc; (c) succinic anhydride, TEA; (d) LiOH, THF/H_2O.

Scheme 8



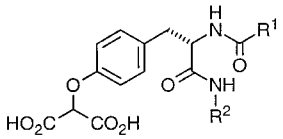
Reagents: (a) Benzyl bromoacetate, $KHCO_3$, acetone or BnO_2CCH_2COCl, pyridine or Ms_2O, pyridine; (b) H_2, Pd/C or LiOH, THF/MeOH; (c) Dimethyl acetylenedicarboxylate or dibenzyl acetylenedicarboxylate, TEA, THF, 50 °C; (d) LiOH, MeOH; (e) H_2, Pd/C.

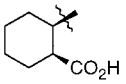
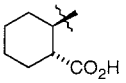
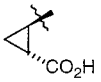
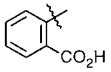
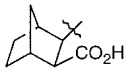
to generate **36**, the esters could be directly removed via saponification to afford diacid **37**, or the amine could be capped with succininc anhydride, leading to triacid **38** after final saponification. Careful comparison of the ^{13}C NMR spectrum of **38** with the corresponding diastereromer **110** prepared from (*R*)-*p*-benzoylphenylalanine established that only a single diastereomer was present within the limits of detection. This important observation confirmed that no racemization of either chiral center was occurring under the saponification conditions.

Scheme 8 summarizes the preparation of several additional tyrosine phoshate bioisostere analogues of **5**. The phenol of **6** could be acylated with benzyl malonyl chloride and subsequently hydrogenated to provide the acyl malonate derivative **40**. Simple alkylation of phenol **6** with benzyl bromoacetate followed by hydrogenation gave the carboxymethyl analogue **41**. Sulfonylation of the phenol with methanesulfonic anhydride was employed to obtain the methylsulfonyl analogue **42**. Homologated malonate analogues were prepared by conjugate addition of phenol **6** or **39** to either dimethyl or dibenzyl acetylenedicarboxylate. The dimethyl maleate **44** was directly saponified to the unsaturated analogue **45**, whereas the dibenzyl maleate **43** was hydrogenated to afford the saturated analogue **46**.

Replacement of the phenolic oxygen of malonate analogue **8** with carbon is outlined in Scheme 9. Palladium-catalyzed olefination of the triflate derived from phenol **3** followed by ozonolysis afforded aldehyde **47**.[23] Knoevenagel condensation installed the unsaturated malonate moiety of **48**. Following amine deprotection and acylation with succinic anhydride, diester **49** could

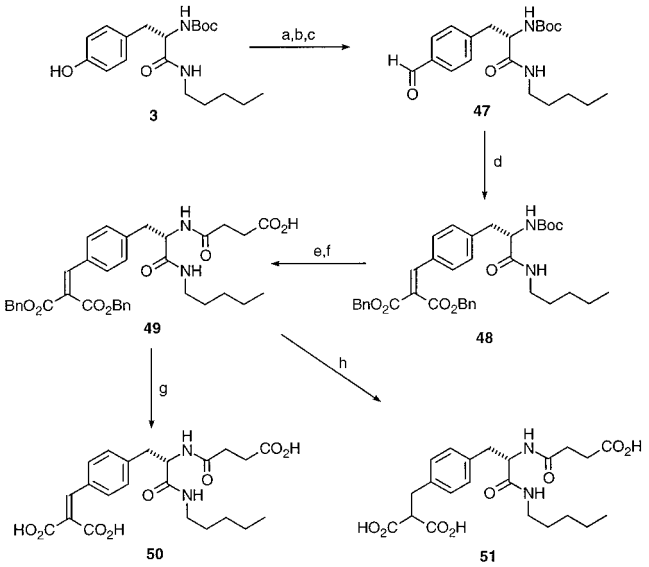
Table 2. N- and C-Terminal Analogues



			% Inhibition of Phosphatases[a] (K_i)			
Cmpd.	R^1	R^2	PTP1	PTP1B	LAR	SHP-2
8	$CH_2CH_2CO_2H$	n-C_5H_{11}	76 (12 μM)	80 (11 μM)	2	8
75	$CH_2CH_2CO_2Bn$	n-C_5H_{11}	39			
76	$CH_2CH_2CONH_2$	n-C_5H_{11}	51			
77	$CH_2CH_2CH_2CO_2H$	n-C_5H_{11}	67		7	10
78	$CH_2OCH_2CO_2H$	n-C_5H_{11}	53		5	8
79	CH_2CO_2H	n-C_5H_{11}	74		7	13
80	$CH_2C(Me)_2CH_2CO_2H$	n-C_5H_{11}	67		6	16
81	$CH_2C(c\text{-}CH_2)_4CH_2CO_2H$	n-C_5H_{11}	72		9	18
82		n-C_5H_{11}	62		8	13
83		n-C_5H_{11}	72		7	10
84		n-C_5H_{11}	57		6	10
85		n-C_5H_{11}	63		10	19
86		n-C_5H_{11}	37		8	5
87	$CH_2CH_2CO_2H$	n-C_6H_{13}	74		5	8
88	$CH_2CH_2CO_2H$	n-C_4H_9	37		5	1
89	$CH_2CH_2CO_2H$	n-$C_{10}H_{21}$	51		4	9
90	$CH_2CH_2CO_2H$	$CH_2(c\text{-}C_6H_{11})$	73		5	12
91	$CH_2CH_2CO_2H$	$CH_2CH_2CH(Me)_2$	48		5	7
92	$CH_2CH_2CO_2H$	$(CH_2)_3Ph$	72		6	12
93	$CH_2CH_2CO_2H$	CH_2(1-napthyl)	61		10	12
94	$CH_2CH_2CO_2H$	$(CH_2)_2CH(Ph)_2$	45		11	9
95	$CH_2CH_2CO_2H$	$CH_2CH(OEt)_2$	46		3	11
96	$CH_2CH_2CO_2H$	CH_2CH_2(4-SO_2NH_2-Ph)	63		5	17
97	$CH_2CH_2CO_2H$	CH_2CH_2(4-OH-Ph)	43			
98	$CH_2CH_2CO_2H$	CH_2(4-CO_2H-Ph)	56		2	58
99	$CH_2CH_2CO_2H$	CH_2(4-CF_3-Ph)	66		4	15
100	$CH_2CH_2CO_2H$	CH_2(3,4-OCH_2O-Ph)	63		6	12
101	$CH_2CH_2CO_2H$	CH_2CH_2OPh	57		4	34
102	$CH_2CH_2CO_2H$	2-(1-piperidinyl)ethyl	23			1
103	$CH_2CH_2CO_2H$	3-(4-morpholinyl)propyl	35		0	2
104	$CH_2CH_2CO_2H$	3-(1-imidazolyl)propyl	26		3	1
105	$CH_2CH_2CO_2H$	(S)-Nle-NH_2	93 (2.0 μM)		4	48
106	$CH_2CH_2CO_2H$	(S)-leucinol	57		0	10

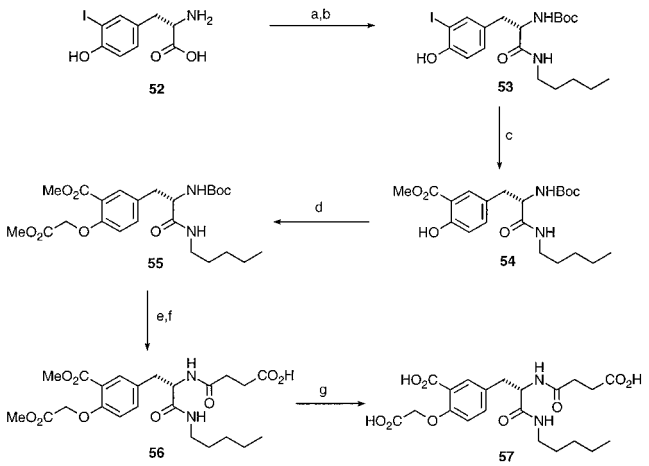
[a] Assayed at 100 μM.

Scheme 9



Reagents: (a) Tf_2O, pyridine; (b) vinyl tributyltin, $(Ph_3P)_2PdCl_2$, LiCl, DMF, 90 °C; (c) O_3, Me_2S; (d) dibenzyl malonate, piperidine, HOAc; (e) HCl/HOAc; (f) succinic anhydride, TEA, (g) LiOH, THF, MeOH; (h) H_2, Pd/C.

Scheme 10



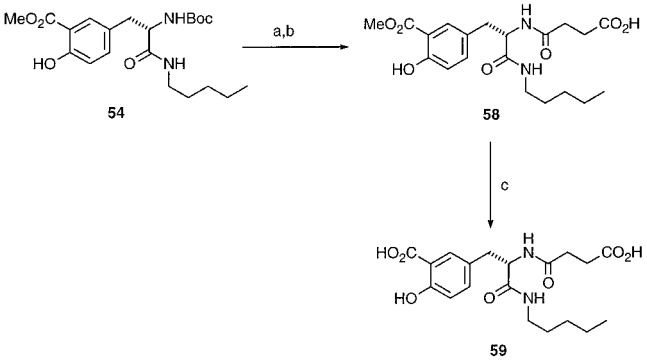
Reagents: (a) Boc_2O, aq NaOH, dioxane; (b) $C_5H_{11}NH_2$, EDC; (c) CO, $Pd(OAc)_2$, DPPF, TEA, DMF/MeOH, 70 °C; (d) methyl bromoacetate, K_2CO_3, acetone; (e) TFA/CH_2Cl_2; (f) succinic anhydride, TEA; (g) LiOH, THF/MeOH.

be directly saponified to unsaturated triacid **50** or hydrogenated to saturated triacid **51**.

Salicylate analogue **57** was prepared as illustrated in Scheme 10. Commercially available *m*-iodotyrosine **52** was Boc-protected and converted to the *n*-pentylamide **53** under standard conditions. Palladium-catalyzed carboxylation of the iodide in methanol afforded methyl ester **54**. The phenol was alkylated with methyl bromoacetate under basic conditions, and the resulting diester **55** was N-acylated with succinic anhydride after cleavage of the Boc group. Monoacid **56** was then saponified to afford triacid **57**. A simpler salicylate analogue **59** could be obtained from **54** by omitting the phenol alkylation step (Scheme 11).

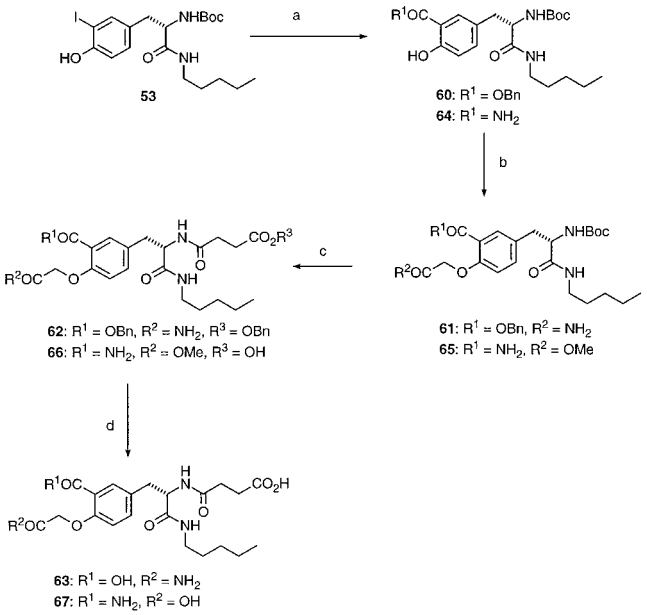
Monoamide analogues of **57** were prepared as illustrated in Scheme 12. Palladium-catalyzed carboxylation of **53** in the presence of benzyl alcohol provided benzyl ester **60**. Alkylation of the phenol with 2-bromoacetamide gave monoamide **61**, which was N-deprotected and N-acylated with monobenzyl succinate, affording diester **62**. Hydrogenolysis then provided the target acetamido diacid **63**. Benzamide analogue **67** was obtained by a similar route. Carbonylation of iodide **53** in the presence of hexamethyldisilazane followed by hydrolysis with aq HCl gave benzamide **64**.[24] Phenol alkylation with methyl bromoacetate, N-deprotection, and N-acylation as before gave benzamide monoester **66**. Final saponification then afforded benzamide diacid **67**.

Scheme 11



Reagents: (a) TFA/CH_2Cl_2; (b) succinic anhydride, TEA; (c) LiOH, THF/MeOH.

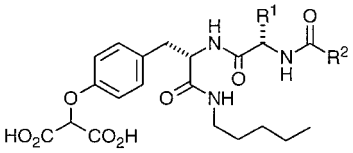
Scheme 12



Reagents: For top, **53**−**63**: (a) CO, $Pd(OAc)_2$, DPPF, BnOH, 80 °C; (b) 2-bromoacetamide, K_2CO_3, acetone, 40 °C; (c) (i) TFA/CH_2Cl_2, (ii) benzyl hydrogen succinate, EDC, DMF; (d) H_2, Pd/C, MeOH. For bottom **53**−**67**: (a) (i) CO, $Pd(OAc)_2$, DPPP, $(Me_3Si)_2NH$, DIPEA, DMF, 90 °C, (ii) HCl/H_2O; (b) methyl bromoacetate, K_2CO_3, acetone; (c) (i) TFA/CH_2Cl_2, (ii) succinic anhydride, TEA; (d) LiOH, THF/MeOH.

Insertion of an amino acid residue into **57** is summarized in Scheme 13. The overall route is analogous to that presented in Schemes 5 and 7. Diester **55** was N-deprotected prior to coupling with Boc-L-phenylalanine to generate **68**. Direct saponification provided diacid **69**. Alternatively, the amine could be deprotected and acylated with monomethyl succinate. The resulting triester **70** was then saponified to afford the target triacid **71**.

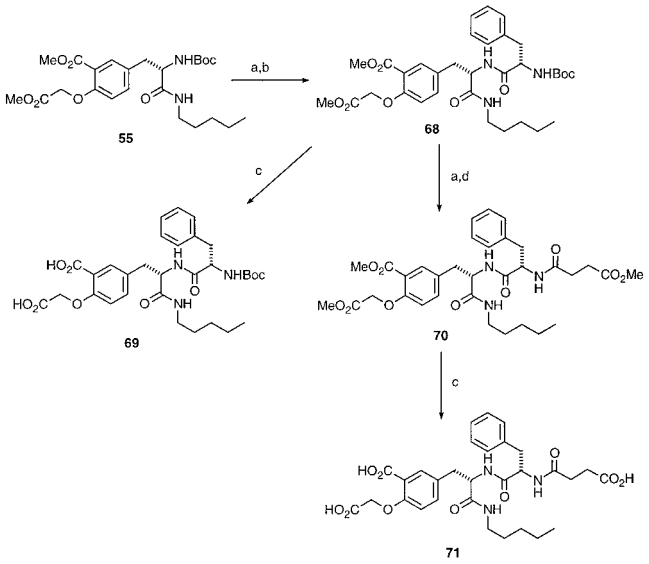
Table 3. Dipeptide Analogues



			% inhibition of phosphatases[a] (K_i)			
cmpd	R^1	R^2	PTP1	PTP1B	LAR	SHP-2
32	CH_2CO_2H	O-tBu	90 (6.0 μM)		10	12
34	CH_2CO_2H	$CH_2CH_2CO_2H$	96 (2.0 μM)	95	7	25
35	CH_2CO_2H	n-C_5H_{11}	91		10	37
37	CH_2(4-Bz-Ph)	O-tBu	91 (20 μM)		24	11
38	CH_2(4-Bz-Ph)	$CH_2CH_2CO_2H$	98 (1.2 μM)	98 (0.7 μM)	26	71
107	$CH_2CH_2CO_2H$	$CH_2CH_2CO_2H$	96		10	28
108	$CH_2CH_2CO_2H$	O-tBu	83		11	14
109	CH_2CO_2H	OCH_2Ph	90		6	50
110	(*R*)-CH_2(4-Bz-Ph)	$CH_2CH_2CO_2H$	54		6	11
111	CH_2(4-BnOPh)	$CH_2CH_2CO_2H$	98 (1.6 μM)		28	33
112	CH_2(4-(2,5-Cl_2Bn)OPh)	$CH_2CH_2CO_2H$	98		33	28
113	CH_2(4-MeOPh)	$CH_2CH_2CO_2H$	98		22	33
114	CH_2Ph	$CH_2CH_2CO_2H$	97 (1.2 μM)	99	23	44
115	CH_2(4-(2,5-Cl_2Bn)OPh)	O-tBu	85		27	45
116	CH_2(4-MeOPh)	O-tBu	90		30	9
117	CH_2Ph	O-tBu	88 (11 μM)	91 (9.0 μM)	27	10
118	CH_3	O-tBu	76 (21 μM)	83	0	3

[a] Assayed at 100 μM.

Scheme 13



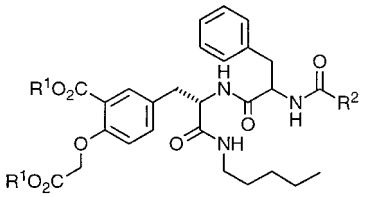
Reagents: (a) HCl/dioxane; (b) Boc-L-Phe-OH, EDC, HOBt, TEA, CH_2Cl_2; (c) LiOH, THF/H_2O; (d) methyl succinate, EDC, HOBt, TEA, CH_2Cl_2.

Results and Discussion

All new compounds were tested for their ability to inhibit a C-terminal truncated, soluble form of recombinant rat PTP1 and/or the equivalent soluble form of recombinant human PTP1B, using *p*-nitrophenyl phosphate (pNPP) as substrate, as previously described.[19] Compounds were also tested for inhibition of other recombinant, purified PTP enzymes as an initial assessment of their specificity. Two PTP enzymes that are structurally dissimilar to PTP1B (LAR, a membrane-spanning PTP, and SHP-2, an SH2 domain-containing PTP involved in insulin signaling[36]) and a dual specificity phosphatase, cdc25b, were utilized as representatives of three major classes of enzymes with PTP activity.[19] 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, all the PTP1B inhibitors tested inhibited cdc25b activity <30% at 100 μM, and none exhibited significant selectivity between PTP1B and TC-PTP. Where inhibition kinetics were determined, plots of V versus S at various concentration of inhibitor were generated and K_i values were computed using the direct linear method of Cornish−Bowden. These data are summarized in Tables 1−4.

We reported recently the discovery that the simple sulfotyrosyl-containing tripeptide **1** retains essentially all of the activity (K_i = 5 μM) of related octapeptidic PTP1B inhibitors.[19] This encouraged us to embark on a peptidomimetic program wherein the peptidic characteristics of **1** would be attenuated and its chemical stability bolstered by replacement of the labile sulfate with a suitable bioisostere. A logical first step was the removal of the terminal amides, resulting in simple bis-(amide) tyrosine sulfate **5** that retained significant, albeit reduced, activity (K_i = 30 μM, Table 1) against PTP1. Subsequent replacement of the tyrosine sulfate moiety of **5** with an *O*-malonate (**8**) was based on the pioneering work of Sikorski,[25] who first demonstrated that *O*-malonate could serve as a surrogate for phosphate, and that of Burke,[26] who had already demonstrated that *O*-malonyltyrosine could be successfully incorporated as a tyrosine phosphate mimic into hexapeptidic inhibitors of PTP1B. In fact, malonate **8** proved to be an even more effective inhibitor than **5** and continued to display competitive inhibition kinetics with a K_i of 11 μM against PTP1B. *Of particular interest was the observation that this simple low molecular weight bis-(amide) malonate inhibited PTP1B with approximately 10-fold greater potency than it inhibited either LAR or SHP-2*. As anticipated, the corresponding diester **73** was

Table 4. *O*-Carboxymethyl Salicylic Acid Analogues



			% inhibition of phosphatases (K_i)				% ctrl 2-DOG uptake[b,c]	
cmpd	R^1	R^2	PTP1[a]	PTP1B[a]	LAR[b]	SHP-2[b]	basal	+ins
68 (*S*,*S*)	CH_3	OtBu		0	0	1	114	72
69 (*S*,*S*)	H	OtBu	84 (1.1 μM)	78	0	2	81	108
70 (*S*,*S*)	CH_3	$CH_2CH_2CO_2CH_3$		0	3	0	225	77
71 (*S*,*S*)	H	$CH_2CH_2CO_2H$		93 (0.22 μM)	0	10	105	83
119 (*S*,*R*)	H	OtBu		19	0	0	107	89

[a] Assayed at 10 μM. [b] Assayed at 100 μM. [c] Uptake of 2-deoxyglucose into L-6 myocytes expressed as a percent of the control. Measurements were taken in the absence of insulin (basal) or in the presence of submaximal concentrations of insulin (+ins).

devoid of inhibitory activity, suggesting that the mode of binding was very similar to that of larger peptides.

A survey of other potential sulfate or malonate bioisosteres is presented in Table 1. Deleting one of the malonate carboxyl groups (**41**) or attaching the malonate to the tyrosine through one of the acyl groups (**40**) obliterated activity. Homologation of the malonate (**45** and **46**) was less deleterious but attenuated activity nonetheless. Attempted replacement of the phenolic oxygen with methylene or methine (**51** and **50**) also reduced inhibitory activity. This latter observation was further support for a peptidic binding mode, as it is known that the phenolic oxygen of tyrosine phosphate in a peptidic substrate participates in key hydrogen-bonding interactions with an ordered water molecule.[27] Overall, our results are consistent with those of Burke et al., who have recently reported that related monocarboxy analogues and analogues replacing the phenolic oxygen with carbon are relatively ineffective inhibitors of PTP1B.[16a] Installation of a fluorine ortho to the *O*-malonate was undertaken to possibly enhance hydrogen-bonding interactions with ordered water (**20**); however, no augmentation of activity was observed. In fact, even perfluorination of the *O*-malonyltyrosine aromatic ring (**21**) was ineffective at significantly improving inhibitory potency. Methanesulfonate and trifluoromethanesulfonate analogues **42** and **74** represent attempts to mimic the sulfate of **5** with neutral functionality. Neither had any significant activity, reinforcing the widely accepted belief that the active site of PTP1B will only recognize negatively charged molecules.

An investigation of analogues incorporating a modified tyrosine scaffold was also undertaken to determine if the natural tyrosine template was optimal (Table 1). As expected, the configuration of the chiral center was critical, as indicated by the significantly lower activity of the enantiomeric analogue **72**. Lengthening the benzyl tether between the aromatic ring and the bis(amide) backbone (**19**) by a single methylene abolished activity. This is an intriguing finding in light of a recent observation by Burke that peptidomimetic inhibitors of PTP1B may be somewhat limited in potential by the depth to which they can insert into the phosphotyrosine binding pocket.[16a] In our case, homologating the tyrosine moiety by one methylene unit was not beneficial, although in principle it should have allowed greater access of the aromatic ring and carboxyl groups to the phosphate binding pocket.

The most productive modification of the tyrosine ring entailed transference of one of the malonate carboxyl groups onto the meta position of the aromatic ring (**57**), which resulted in a marked improvement in potency, particularly against PTP1 (Ki = 2.8 μM) (Table 1). The Burke group[28] independently reported incorporation of this novel tyrosine phosphate bioisostere into hexapeptides at about the same time as our discovery,[29] and Iversen and Andersen et al.[30] have recently reported a similar tyrosine phosphate bioisostere, 2-(oxalylamino)-benzoic acid. We found that the new aromatic carboxylic acid moiety itself was not sufficient for inhibitory activity, since removal of the *O*-carboxymethyl group obliterated activity (**59**). In fact, simple masking of either carboxyl of **57** as the corresponding primary carboxamide (**63**, **67**) was similarly detrimental, establishing the critical importance of having both carboxyl groups present.

Although it was expected that the bis(amide) backbone would eventually need to be modified to achieve acceptable pharmacokinetic properties, we decided to initially retain this functionality to facilitate rapid modification of the N- and C-termini. Table 2 summarizes our initial work in this area. On the basis of the prevailing peptidic SAR, it was assumed that the carboxyl group of the N-terminus would be required, since it corresponded to the P-1 aspartic acid present in the best peptidic PTP1B inhibitors. This was confirmed with ester and amide analogues **75** and **76**, each of which had substantially attenuated activity relative to acid **8**. A series of carboxylic acids was thus generated off the N-terminus (**77**−**86**) to investigate the effect of chain length, substitution, and conformational restriction. Unfortunately, none of the new analogues exhibited improved activity relative to the simple succinic acid of **8**. Attention was then focused on the C-terminal *n*-pentyl amide. Chain length, simple substitution, and incorporation of heteroatoms or aromatic rings were all investigated (**87**−**106**). To our surprise, although removal of a single methylene unit was quite detrimental to activity (**88**), larger groups were not more effective (e.g., **87**, **89**, **90**), suggesting that a shallow pocket of defined length was being accessed. Consistent with this hypothesis was the lack of improvement with the

addition of aromatic ring(s), both substituted and unsubstituted. Preliminary attempts to add hydrophilic functionality (e.g., **96**, **102**−**104**) were even less encouraging.

It was of considerable interest to us to confirm that our small malonate PTP1B inhibitors bound in a mode similar to that of larger peptidic tyrosine phosphate substrates, because enzyme/substrate structural information was available that might guide further analogue design. Barford had recently published an X-ray structure of a catalytically inactive C215-S mutant of PTP1B bound to the hexapeptidic substrate DADEpYL-NH_2.[27] Recognizing that **8** was highly analogous to the central tripeptide (EpYL) of this substrate, it was logical to assume that introduction to **8** of either a C-terminal carboxamide or a second N-terminal carboxylic acid analogous to the hexapeptidic P-2 aspartic acid ought to augment binding, as both of these functionalities were reported by Barford to make hydrogen-bonding interactions with the enzyme. Analogues **105** (Table 2) and **34** (Table 3) were prepared to test these assumptions, and each of these analogues indeed proved to be significantly more active ($K_i = 2\ \mu$M) than **8** ($K_i = 12\ \mu$M). Although these analogues were now the most active to date and useful for supporting the proposed mode of binding, neither could be considered a step forward toward a viable drug. An attempt to replace the C-terminal primary carboxamide of **105** with hydroxyl (**106**) unfortunately attenuated activity. Fortunately, we subsequently noted that the terminal carboxylic acid moiety of **34** was not absolutely required for effective inhibition of PTP1. The simple *N*-Boc derivative **32** remained more active than **8**, suggesting that the new amide moiety was in part responsible for the improved binding affinity. *N*-Cbz and *N*-alkanoyl derivatives **109** and **35** indeed were similarly active (Table 3).

A second literature report provided a clue toward further improving the physical properties of **34** without sacrificing activity. Zhang reported that replacement of the P-1 glutamic acid residue of PTP1B substrate AcDADEpYLIPQQG with the photolabile amino acid *p*-benzoylphenylalanine (Bpa) did not significantly alter its susceptibility to dephosphorylation by PTP1, a surprising observation in light of the markedly different physical properties of the Bpa residue relative to glutamic acid.[31] Therefore, analogue **38** was prepared to test whether a similar replacement of the aspartic acid of **34** with L-Bpa would be viable. Importantly, we observed an increase in potency, achieving for the first time submicromolar activity against PTP1B ($K_i = 0.7\ \mu$M) (Table 3). As expected, the configuration of the new chiral center was important, as the corresponding analogue prepared from D-Bpa (**110**) was approximately 2 orders of magnitude less active. Subsequent SAR quickly determined that simple phenylalanine analogue **114** was just as effective as **38** and had the advantage of substantially lower molecular weight. Paring further down to the alanine derivative **118** eroded activity. Of particular interest was the improvement in specificity for PTP1 vs SHP-2 of **114** relative to **38** (Table 3). This at least suggests that higher lipophilicity may be detrimental to selectivity, a conclusion that is supported by other comparisons in this work (e.g., **109** is less specific for PTP1 than is **32**; **115** is less specific than **116**), and that is consistent with the known preference of SHP-2 for peptidic substrates with lipophilic residues N-terminal to the phosphotyrosine.[37] The corresponding *N*-Boc derivatives again retained activity (**37**, **115**−**118**), demonstrating for the first time in this series that effective inhibition of PTP1 could be achieved in the absence of an N-terminal carboxyl group, a finding of obvious importance from a drug development standpoint.

Table 4 summarizes biological data for analogues that combine the most active tyrosine phosphate bioisostere (from **57**) with the "optimized" N-terminus of **114**. The two elements proved to be complementary, as the resulting analogue **71** inhibited PTP1B with a potency exceeding either of the forerunners ($K_i = 0.22\ \mu$M). *Of particular interest was the observation that this improved potency vs PTP1B was associated with an essentially complete loss of activity against LAR and SHP-2.* The simple (*S*,*S*)-*N*-Boc derivative **69** also benefited from the new tyrosine phosphate bioisostere, achieving a K_i of 1.1 μM, the most potent analogue of this series lacking any N-terminal carboxyl group. The relative configuration of the chiral centers was again confirmed to be important by the markedly lower potency of the analogue **119** prepared from (*R*)-Boc-Phe.

Unfortunately, none of the new analogues exhibited reproducible augmentation of insulin-stimulated 2-deoxyglucose (2-DOG) uptake into intact cells (L6 myocytes, 3T3-L1 adipocytes, primary rat adipocytes) (representative data presented in Table 4). This is not altogether surprising, considering the impediment to cell penetration that would be expected to result from the charge associated with multiple carboxyl groups. In fact, it was demonstrated that a number of analogues from this series are extremely poor at penetrating a Caco-2 cell monolayer,[32] with permeability coefficients generally less than 5 nm/s (data not shown). Prodrug esters were investigated as a logical recourse, two of which (**68** and **70**) indeed exhibited greatly enhanced penetration (permeability coefficients of 680 and 136 nm/s, respectively). Although **68** remained inactive at stimulating 2-DOG uptake into L-6 myocytes, **70** elicited an approximate doubling of 2-DOG uptake at a concentration of 100 μM (Table 4). Compound **70** caused a large increase in basal 2-DOG uptake by L6 myocytes, and this is consistent with the hypothesis that, in the absence of insulin, the insulin receptor tyrosine kinase is not entirely quiescent and PTP1B is required to dampen spontaneous signaling. The large effect of **70** on basal 2-DOG uptake was subtracted when the effect of **70** on insulin-dependent 2-DOG uptake was computed, and this results in an apparent lack of effect of **70** in the presence of insulin. In fact, in a typical experiment ($n = 3$) in which **70** was tested in this way, uptake in the presence of insulin (10 nM) but absence of **70** [50.6 ± 0.5 pmol min^{-1} (mg of protein)$^{-1}$] was increased by **70** to a value [72.7 ± 0.3 pmol min^{-1} (mg of protein)$^{-1}$] that was similar to the maximal observed with insulin (300 nM) alone [79.5 ± 1.3 pmol min^{-1} (mg of protein)$^{-1}$].

The effect of **70** on 2-DOG uptake was accompanied by increased levels of IR and IRS-1 and -2 phosphorylation,[19] evidence that inhibition of PTP1B was occurring within the cell, even though **70** itself is devoid of

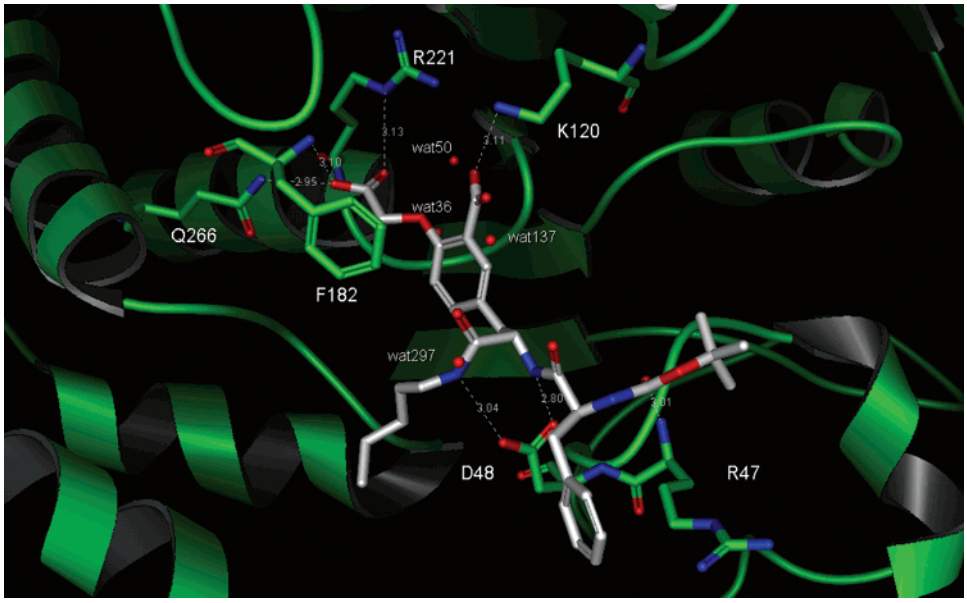


Figure 1. X-ray structure (2.2 Å resolution) of **69** (white) bound to PTP1B (green). The enzyme backbone is shown as a ribbon diagram with key residues presented in detail and labeled. Heteroatoms are colored by type (O, red; N, blue). Bound water molecules are presented as red spheres. Selected hydrogen-bonding interactions with enzyme are depicted with dashed lines (distances in angstroms).

inhibitory activity. Triester **70** also augmented 2-DOG uptake into and IR and IRS-1 phosphorylation of an insulin-resistant 3T3-L1 adipocyte model.[19] The triester is likely cleaved enzymatically in cells to an active component(s), because we demonstrated time-dependent hydrolysis of **70** to a mixture of mono- and diacids by porcine hepatic esterase (no triacid **71** was detected). Although not all of the components of this mixture could be identified unambiguously without independent syntheses, the mixture itself was shown to be active at inhibiting PTP1B, and inhibition was proportional to the amount of diacid product.[19]

The mode of binding of triacid **71** was eventually confirmed by solving an X-ray cocrystal structure with a C-terminal-truncated form of PTP1B.[19] Since dissection of the N-terminal carboxyl group was likely to be a requirement for further development of this series, it was important to determine what effect, if any, this structural modification would have on the binding mode. Thus, **69** was selected for cocrystallization studies with PTP1B. The overall structure of the PTP1B compound **69** complex proved to be very similar to the previously reported structure of PTP1B with compound **71**[19] (Figures 1 and 2; coordinates have been deposited with the PDB under the file name 1JF7). This was not unexpected, as these two compounds only differ in the nature of their N-terminal substituent. As with **71**, the binding of compound **69** is accompanied by closure of the WPD loop (comprising of residues 179−189) of the enzymes catalytic site. The peptide backbone portion of both bound inhibitors forms an extended β-strand conformation closely resembling that seen previously for peptidic substrates.[27] These compounds maintain two conserved hydrogen bonds between the Asp48 side chain and the main chain nitrogens on either side of the central *O*-carboxymethyl salicylic acid tyrosine phosphate mimic (P and P + 1 positions) and a third between the main chain nitrogen of Arg47 and the Boc carbonyl of the inhibitor at the P-1 position. The phenyl ring of the phosphotyrosine (pTyr) mimic also makes hydrophobic

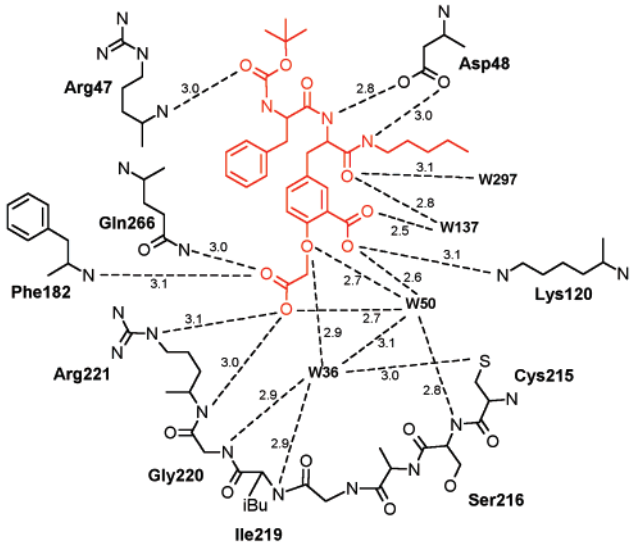


Figure 2. Schematic representation of the hydrogen-bonding interactions between **69** (red) and PTP1B (black). Bound water molecules are labeled as W. All distances are in angstroms, and only interactions of 3.1 Å or less are shown.

packing interactions with the side chains of Tyr46, Val49, Ala217, Ile219, Gln262, and Phe181. In addition, the C-terminal pentyl chains (P + 1) of both compounds lay in a shallow hydrophobic pocket and form hydrophobic contacts with the side chains of Val49, Ile219, and Gln262.

In the PTP1B-pTyr peptide crystal structures, two of the phosphate oxygen atoms of the pTyr residue make two hydrogen bonds, each to the main chain amides of Ser216, Ala217 and Ile219, Gly220, respectively.[27] The phosphate mimic headgroups of both compounds **69** and **71** are positioned somewhat closer to the WPD loop than the phosphate group of the substrate, resulting in the loss of all four hydrogen bonds. These interactions with the backbone amides are instead formed by new structurally bound water molecules. These solvent molecules

form a network of hydrogen bonds between residues of the phosphate binding site (the amides of Ser216, Ile219, and Gly220, the NH1 of Arg221) and the ether oxygen of *O*-carboxymethyl salicylic acid headgroup itself (Figure 2). The positioning of the headgroup closer to the WPD loop also results in the displacement of a bound water molecule hydrogen bonded to Gln266 in the PTP1B−substrate structures with compensatory hydrogen bonds being formed by headgroup oxygens (Figure 2).

The terminal carboxylate of the *O*-carboxymethyl salicylic acid headgroup makes a series of five hydrogen bonds to the main chain nitrogen of Phe182, the side chain amide of Gln266, the side chain NE1 and main chain nitrogen of Arg221, and to one of the ordered waters located in the phosphate binding site. The *o*-carboxyl substituent of the inhibitor in turn forms hydrogen bonds to the side chain of Lys120 (3.1 Å) and to two water molecules, including the one mentioned above. However, there is no significant interaction observed between the *o*-carboxylate and the side cain of Asp181 as was apparent in the PTP1B−**71** complex, where they share a proton. This difference is probably due to the fact that the crystals of the PTP1B−**69** complex were grown at a somewhat higher pH (8.0 vs 6.5), resulting in the Asp181 side chain carboxylate being unprotonated and thus unable to interact with the inhibitor.

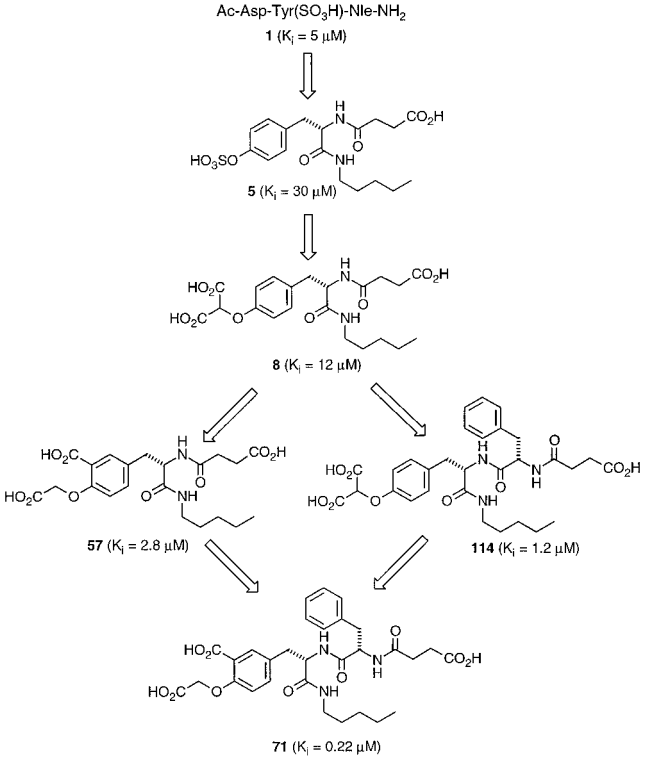
The only other significant difference in binding between compounds **69** and **71** is observed at the N-terminus, where the P-2 carboxylic acid function of compound **71** is replaced by a hydrophobic *N*-Boc group. As a consequence of the loss of a hydrogen bond between the terminal carboxylate of compound **71** and the Arg47 side chain, there is observed a concerted repositioning of both Arg47 and P-1 Phe side chains by about 3 Å. Yet despite this movement, both the hydrophobic interactions between these two side chains and the hydrogen bond between the P-1 carbonyl and the Arg47 main chain amide are maintained. This again demonstrates, as has been previously observed,[19,34] the conformational plasticity of the Arg47 side chain to accommodate a variety of N-terminal ligand structures.

Although there are two PTP1B molecules in the crystal unit cell, only one was seen to have a bound inhibitor. The binding site of the other PTP1B molecule is effectively blocked by crystal contacts from a symmetry-related PTP1B molecule. In particular, amino acids 236−240 in the symmetry-related enzyme make similar contacts with the binding site to those seen in the peptide substrate crystal structures. The symmetry related Lys233 is positioned equivalently to a substrate phosphotyrosine and is held in place by hydrogen bonds from the binding site Asp48 to the backbone amides of Lys233 and Asp240. The lysine, however, does not extend down to the phosphate cradle, which is occupied by a sulfate ion, but instead hydrogen bonds to the side chain of Gln262.

Conclusions

Directed screening of the Pharmacia compound collection for reversible competitive inhibitors of PTP1B bearing potential phosphotyrosine mimics resulted in the identification of sulfotyrosyl-containing octapeptides

Scheme 14. Key Analogues in the Evolution of the Tripeptide PTP1B Inhibitor 1



with K_i values in the range of 5 μM. Inhibitory activity was subsequently determined to reside unexpectedly within the N-terminal tripeptidic sequence Ac-Asp-Tyr-(SO_3H)-Nle-NH_2 ($K_i = 5\ \mu$M). An analogue program was launched to attenuate the peptidic character of this lead and to enhance its potency. Modifications in the early phase of this work included a survey of phosphate mimics, alteration of the core tyrosine template, N- and C-terminal replacements with non-amino acid components, and insertion of specific amino acids at the P-1 position to evaluate the mode of binding. Key analogues that guided the evolution of the SAR are summarized in Scheme 14. The most significant analogue arising from this work was **71**, which possesses submicromolar affinity for PTP1B ($K_i = 0.22\ \mu$M) without significantly inhibiting LAR or SHP-2 at concentrations up to 100 μM. Furthermore, it was demonstrated that a triester prodrug of this molecule (**70**) enhances insulin signaling in two different cell lines. Key elements of our SAR study that could guide future inhibitor design in this area include the following: confirmation of the remarkable effectiveness and PTP1B specificity of the novel tyrosine phosphate mimic *O*-carboxymethyl salicylic acid, demonstration that the tyrosine template is optimal relative to a number of closely related structures, successful replacement of the P-1 aspartic acid present in peptidic inhibitors with the neutral and lipophilic phenylalanine residue, and the observation that the N-terminal carboxyl group can be jettisoned altogether with retention of significant activity. Solution of the X-ray cocrystal structure of the neutral N-terminal analogue **69** bound to PTP1B revealed that the (Boc)-Phe N-terminus interacts with the enzyme primarily through the urethane carbonyl group and through hydrophobic interactions with the aromatic ring. Additional SAR studies encompassing more potent neutral

N-terminal analogues, bioisosteric replacement of the *O*-carboxymethyl salicylic acid carboxyl groups, and alteration of the bis(amide) backbone will be the subject of future reports.

Experimental Section

All melting points (mp) were obtained on a capillary melting point apparatus and are uncorrected. Proton magnetic resonance spectra were recorded on a Bruker AM-300 or AM-400 spectrometer in the deuterated solvents indicated. Chemical shifts were recorded in parts per million (δ scale) and are reported relative to internal tetramethylsilane. ^{13}C NMR spectra were recorded on a Bruker AM-300 spectrometer at 75.4 MHz. Flash column chromatography separations were carried out using EM Science silica (mesh 230−400). Electron impact (EI) mass spectra were obtained with an ionization voltage of 70 eV. Alternatively, ionization was achieved by fast atom bombardment (FAB) or electrospray (ES+ or ES−). Reagents and solvents were purchased from common suppliers and were used as received. All nonaqueous reactions were run under a nitrogen atmosphere. All starting materials were commercially available unless otherwise noted.

***tert*-Butyl (1S)-1-(4-Hydroxybenzyl)-2-oxo-2-(pentylamino)ethylcarbamate (3).** To a 0 °C mixture of Boc-L-tyrosine (2.04 g, 7.25 mmol) and amylamine (0.93 mL, 7.98 mmol) in methylene chloride (30 mL) was added 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide (EDC) (1.53 g, 7.98 mmol). The white mixture was stirred at 0 °C for 5 min and at room temperature for 23 h. The resulting solution was diluted with methylene chloride (30 mL) and washed successively with 0.5 M HCl (40 mL), water (20 mL), and sat. aq sodium bicarbonate (25 mL). The organic phase was dried over magnesium sulfate and concentrated to a foam (1.84 g, 73%) that was sufficiently pure to carry into the next step. An analytical sample could be obtained by flash chromatography (1/1 ethyl acetate/hexane) as a glass: ^{1}H NMR ($CDCl_3$) δ 7.01 (d, J = 7 Hz, 2 H), 6.74 (d, J = 7 Hz, 2 H), 5.90 (bt, 1 H), 5.17 (bd, 1 H), 4.21 (bq, J = 7 Hz, 1 H), 3.13 (m, 2 H), 2.93 (m, 2 H), 1.41 (s, 9 H), 1.1−1.4 (m, 6 H), 0.85 (t, J = 7 Hz, 3 H).

4-{[(1S)-1-(4-Hydroxybenzyl)-2-oxo-2-(pentylamino)-ethyl]amino}-4-oxobutanoic Acid (4). To a solution of **3** (5.28 g, 15.1 mmol) in dioxane (40 mL) chilled in an ice bath was added a freshly prepared solution of HCl in dioxane (about 3 M, 25 mL). The solution was then stirred at rt for 1.5 h. The solution was diluted rapidly with ether (350 mL) until no further precipitation was evident. The mixture was stirred vigorously until all insoluble material was adhering to the sides of the flask. After decanting the supernatant, the crude material was taken up in more ether (200 mL) and sonicated until it was a fine solid (required about 1 h). Filtration gave the amine hydrochloride as a hygroscopic white powder (4.32 g, approximately 100%).

Triethylamine (307 μL, 2.2 mmol) was added to a 0 °C mixture of the crude amine hydrochloride from above (287 mg, 1.0 mmol) in methylene chloride (4 mL), causing rapid dissolution. To this solution was added succinic anhydride (100 mg, 1.0 mmol), and the reaction was stirred at room temperature for 25 h. The reaction was then diluted with ethyl acetate (20 mL) and washed successively with 0.5 M HCl (10 mL) and brine (10 mL). The organic phase was dried over magnesium sulfate and concentrated to a viscous oil (350 mg, approx 100%) that solidified on standing: ^{1}H NMR (DMSO) δ 9.1 (s, 1 H), 7.98 (d, J = 7 Hz, 1 H), 7.74 (t, J = 6 Hz, 1 H), 6.96 (d, J = 7 Hz, 2 H), 6.60 (d, J = 7 Hz, 2 H), 4.30 (m, 1 H), 2.9−3.1 (m, 2 H), 2.8 (dd, J = 7, 14 Hz, 1 H), 2.60 (dd, J = 8, 14 Hz, 1 H), 2.3 (m, 4 H), 1.1−1.4 (m, 6 H), 0.83 (t, J = 7 Hz, 3 H). Anal. ($C_{18}H_{26}N_2O_5$)·0.44H_2O) C, H, N.

4-Oxo-4-({(1S)-2-oxo-2-(pentylamino)-1-[4-(sulfooxy)-benzyl]ethyl}amino)butanoic Acid (5). A solution of **4** (100 mg, 0.29 mmol) and pyridine−sulfur trioxide complex (500 mg) in DMF:pyridine (1:1, 10 mL) was stirred under nitrogen at rt for 20 h. NMR analysis of an aliquot indicated complete conversion to product. Solvent was removed under vacuum, leaving a solid that was purified by preparative reverse phase HPLC on a Vydac C-18 column (27.5 × 250 mm) using a water/acetonitrile gradient, each phase containing 0.1% TFA. Clean fractions, as determined by analytical HPLC, were pooled, and the acetonitrile was evaporated under reduced pressure. The aqueous solution was then lyophilized: ^{1}H NMR (DMSO) δ 8.03 (d, J = 8 Hz, 1 H), 7.79 (t, J = 7 Hz, 1 H), 7.06 (d, J = 7 Hz), 7.01 (d, J = 7 Hz, 2 H), 4.33 (m, 1 H), 2.99 (m, 2 H), 2.87 (dd, J = 6, 13 Hz, 1 H), 2.68 (dd, J = 8, 13 Hz, 1 H), 2.3 (m, 4 H), 1.1−1.3 (m, 6 H), 0.83 (t, J = 7 Hz, 3 H); ES MS m/z (negative ion, rel intens) 429 (100), 214 (20); HRMS (FAB) calcd for $C_{18}H_{26}N_2O_8S$ + H 431.1488, found 431.1473.

Benzyl 4-{[(1S)-1-(4-Hydroxybenzyl)-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoate (6). The Boc group of **3** was removed as described in the preparation of **4**. EDC (368 mg, 1.92 mmol) was added to a 0 °C solution of the crude amine hydrochloride (500 mg, 1.74 mmol), monobenzyl succinate (368 mg, 1.92 mmol), and triethylamine (270 uL, 1.92 mmol) in methylene chloride (7 mL). The reaction was stirred at room temperature for 24 h. After dilution with ethyl acetate (40 mL), the mixture was washed successively with 0.5 M HCl, water, and sat. aq sodium bicarbonate (20 mL each). The organic phase was dried over magnesium sulfate and concentrated to a white amorphous solid (694 mg, 91%) that was analytically pure. $[\alpha]^{25}_D$ −5.4° (c 0.017, methanol); ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 5 H), 7.05 (d, J = 8 Hz, 2 H), 6.74 (d, J = 8 Hz, 2 H), 6.21 (d, J = 8 Hz, 1 H), 5.90 (bt, J = 7 Hz, 1 H), 5.68 (bs, 1 H), 5.13, 5.09 (ABq, J = 14 Hz, 2 H), 4.54 (m, 1 H), 3.12 (q, J = 7 Hz, 2 H), 3.06, 2.90 (ABqd, J = 7, 14 Hz, 2 H), 2.4−2.85 (m, 4 H), 1.1−1.4 (m, 6 H), 0.86 (t, J = 7 Hz, 3 H).

Di-*tert*-butyl2-{4-[(2S)-2-{[4-(Benzyloxy)-4-oxobutanoyl]-amino}-3-oxo-3-(pentylamino)propyl]phenoxy}malonate (7). A solution of di-*tert*-butyl diazomalonate[21] (396 mg, 1.63 mmol) in benzene (2 mL) was added over 6 h to an 80 °C mixture of **6** (342 mg, 0.776 mmol) and rhodium(II) acetate (7 mg) in benzene (33 mL) via syringe pump, during which time most of the starting material went into solution. The blue-green solution was stirred at that temperature for an additional hour and then overnight at room temperature. The reaction was filtered through a medium frit and then concentrated in vacuo. Flash chromatography (80 g silica, 70% ethyl acetate/hexane) provided the title material (232 mg, 46%) as a foam: ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 5 H), 7.12 (d, J = 9 Hz, 2 H), 6.89 (d, J = 9 Hz, 2 H), 6.10 (bd, J = 8 Hz, 1 H), 5.81 (bt, J = 7 Hz, 1 H), 5.12, 5.08 (ABq, J = 12 Hz, 2 H), 4.94 (s, 1 H), 4.52 (m, 1 H), 3.1 (m, 3 H), 2.6−2.95 (m, 3 H), 2.45 (m, 2 H), 1.49 (s, 18 H), 1.1−1.4 (m, 6 H), 0.86 (t, J = 7 Hz, 3 H).

2-{4-[(2S)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic Acid (8). Trifluoroacetic acid (5 mL) was added to a solution of **7** (225 mg, 0.344 mmol) in methylene chloride (5 mL) with ice-bath chilling. The solution was stirred at room temperature for 2 h. Concentration in vacuo afforded a light amber foam. This was dissolved in ethyl acetate and concentrated in vacuo three times to get rid of traces of trifluoroacetic acid. The residue was then dissolved in methanol (10 mL) and subjected to three cycles of evacuation and nitrogen purge at 0 °C before the introduction of 10% Pd/C (20 mg). The mixture was then hydrogenated at 1 atm for 1.5 h. The mixture was filtered through Celite and concentrated in vacuo. The crude glass was taken up in methylene chloride (40 mL) and sonicated until it was all suspended. Filtration gave a brittle white amorphous solid (116 mg, 88% overall from **7**): mp 117−120 °C, dec; $[\alpha]^{25}_D$ = −0.7° (c 0.0058, methanol); ^{1}H NMR (CD_3OD) δ 8.13 (bd, J = 8 Hz, 1 H), 7.81 (bt, J = 7 Hz, 1 H), 7.17 (d, J = 8 Hz, 2 H), 6.90 (d, J = 8 Hz, 2 H), 5.23 (s, 1 H), 4.48 (m, 1 H), 3.1 (m, 3 H), 2.81 (dd, J = 8, 14 Hz, 1 H), 2.3−2.6 (m, 4 H), 1.2−1.5 (m, 6 H), 0.89 (t, J = 7 Hz, 3 H); MS (FAB) m/z (rel intensity) 453 (M + H, 47), 453 (47), 238 (15), 194 (17), 136 (12), 133 (17), 101 (12), 88 (99), 86 (30), 55 (15), 43 (22). Anal. ($C_{21}H_{28}N_2O_9$·0.6H_2O) C, H, N.

Benzyl (1S)-1-(4-Hydroxybenzyl)-2-oxo-2-(pentylamino)ethylcarbamate (10b). To a mixture of Cbz-Try-OH (**9b**)-(2 g, 6.3 mmol) in CH_2Cl_2 (75 mL) and DMF (5 mL) at 0 °C

was added EDC (1.21 g, 6.3 mmol). After a few minutes, amylamine (0.74 mL, 6.3 mmol) was added, and the mixture was warmed to room temperature and stirred for 4.5 h. HCl (10%, 50 mL) was added, and the phases were separated. The organic phase was washed with sat. NaCl (30 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (75 g SiO_2, 50% EtOAc/hexane) to give 1.7 g (68%) of a white solid: ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 5 H), 7.06 (d, 2 H), 6.74 (d, 2 H), 5.53 (br s, 1 H), 5.35 (br s, 1 H), 5.09 (s, 2 H), 5.05 (br s, 1 H), 4.25 (q, 1 H), 3.14 (q, 2 H), 3.15 (dd, 1 H), 2.92 (dd, 1 H), 1.14−1.35 (m, 6 H), 0.86 (t, 3 H).

Dibenzyl 2-{4-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonate (11a). To a stirring solution of **3** (2.0 g, 5.71 mmol) in acetone (50 mL) was added K_2CO_3 (1.57 g, 11.42 mmol) at ambient temperature. To the resulting heterogeneous mixture was added dibenzyl bromomalonate (2.89 g, 6.28 mmol), and the mixture was stirred at ambient temperature overnight. The resulting amber suspension was diluted with H_2O (100 mL) and extracted with EtOAc (2 × 100 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed in vacuo. The residue was purified via flash chromatography (2: 1 EtOAc/hexane) to afford 1.26 g (35%) of a white solid: ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H), 1.16−1.38 (m, 8 H), 1.39 (s, 9 H), 2.96 (m, 2 H), 3.13 (dd, 2 H), 4.21 (q, 1 H), 5.20 (s, 4 H), 5.08 (m, 1 H), 5.23 (s, 1 H), 5.85 (t, 1 H), 6.83 (d, 2 H), 7.07 (d, 2 H), 7.25 (m, 10 H).

Diethyl 2-{4-[(2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-oxo-3-(pentylamino)propyl]phenoxy}malonate (11b). To a mixture of **10b** (200 mg, 0.5 mmol) and K_2CO_3 (146 mg, 1.0 mmol) in acetone (1.5 mL) was added diethyl chloromalonate (0.17 mL, 1.0 mmol). The mixture was stirred vigorously for 18 h. The mixture was partitioned between EtOAc (10 mL) and H_2O (5 mL). The organic phase was washed with H_2O and sat. NaCl. After drying ($MgSO_4$), the solvent was removed under reduced pressure. The residue was purified by flash chromatography (40% EtOAc/hexane) to provide 140 mg (49%) of a white solid: ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 5 H), 7.10 (d, 2 H), 6.88 (d, 2 H), 5.55 (t, 1 H), 5.31 (br s, 1 H), 5.14 (s, 1 H), 5.08 (s, 2 H), 4.30 (m, 5 H), 3.12 (m, 3 H), 2.93 (ab q, 1 H), 1.30 (m, 12 H), 0.86 (t, 3 H); MS (ES−) 541. Also obtained off the column was a small amount of the α-chloromalonate **15** (10 mg): ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 5 H), 7.11 (m, 4 H), 5.55 (br s, 1 H), 5.25 (m, 1 H), 5.08 (s, 2 H), 4.31 (m, 4 H), 3.12 (q, 2 H), 2.98 (m, 2 H), 1.10−1.40 (m, 12 H), 0.86 (t, 3 H); MS (ESI+) for $C_{29}H_{37}ClN_2O_8$ *m/z* 577 $(M + H)^+$; MS (ES−) for $C_{29}H_{37}ClN_2O_8$ *m/z* 575 $(M - H)^-$.

Dibenzyl 2-{4-[(2*S*)-2-Amino-3-oxo-3-(pentylamino)-propyl]phenoxy}malonate Hydrochloride (12a). To a stirring solution of **11a** (2.85 g, 4.5 mmol) in HOAc (25 mL) at ambient temperature was added 1.5 N HCl/HOAc (20 mL), and the resulting solution was stirred at ambient temperature for 2 h. The solvent was evaporated to 30 mL, and the solution was triturated with Et_2O (400 mL). The resulting turbid suspension was stirred at ambient temperature for 30 min, sonicated, and filtered to afford 2.50 g (98%) of **12a** as a white solid: ^{1}H NMR (DMSO) δ 0.80 (t, 3 H), 1.18 (m, 8 H), 2.93 (m, 3 H), 3.04 (m, 1 H), 3.87 (t, 1 H), 5.20 (s, 4 H), 5.83 (s, 1 H), 6.91 (d, 2 H), 7.13 (d, 2 H), 7.32 (m, 10 H), 8.39 (t, 1 H) (NH_2 not observed).

Diethyl 2-{4-[(2*S*)-2-Amino-3-oxo-3-(pentylamino)propyl]phenoxy}malonate Hydrochloride (12b). To a solution of **11b** (2.9 g, 5.3 mmol) in absolute EtOH (100 mL) and THF (10 mL) was added 10% Pd/C (0.29 g, moistened with absolute EtOH). The mixture was hydrogenated (40 psi) for 1 h. The mixture was filtered through Celite and concentrated under reduced pressure. The residue was dissolved in a 1 M solution of HCl in HOAc (10 mL). After stirring for several minutes, the mixture was concentrated to 2−3 mL. A large amount of Et_2O was added, and the mixture was cooled to 0 °C. The Et_2O was decanted from the oil, which had settled on the flask. The oil was washed with Et_2O, and the Et_2O was decanted again. To the oil was added Et_2O again, and the mixture was sonicated. The oil gradually crystallized, and the solid was collected to provide 2.0 g (83%) of **12b** as an off-white solid: ^{1}H NMR (DMSO) δ 8.39 (t, J = 7 Hz, 1 H), 8.23 (bs, 3 H), 7.14 (d, J = 9 Hz, 2 H), 6.90 (d, J = 9 Hz, 2 H), 5.63 (s, 1 H), 4.20 (m, 4 H), 3.88 (t, J = 7 Hz, 1 H), 3.05 (m, 1 H), 2.93 (m, 3 H), 1.19 (t, J = 7 Hz, 6 H), 1.1−1.4 (m, 6 H), 0.82 (t, J = 7 Hz, 3 H).

General Procedure for Reaction of 12a with Anhydrides To Afford Amides 13a. To a stirring solution of **12a** (0.20 g, 0.35 mmol) in CH_2Cl_2 (10 mL) was added triethylamine (78 mg, 0.77 mmol) at 0 °C. The requisite anhydride (0.35 mmol) was added in one portion and the resulting solution was allowed to stir for 16 h while warming to ambient temperature. The solution was diluted with CH_2Cl_2 (50 mL) and washed with 10% HCl/H_2O (2 × 50 mL). The combined organic phases were dried over $MgSO_4$ and solvent removed in vacuo to afford material suitable for subsequent transformations.

General Procedure for Hydrogenation of Benzyl Esters 13a To Afford Acids 14. To a solution of the requisite ester in MeOH (∼0.02 M) at ambient temperature was added 10% Pd/C (10 wt %), and the resulting mixture was hydrogenated at atmospheric pressure for 3 h. The mixture was filtered through Celite and solvent removed to afford analytically pure material.

Representative Procedure for Conversion of 12b to 14. See procedure for compound **76**.

2-{4-[3-[(3-Carboxypropanoyl)amino]-4-oxo-4-(pentylamino)butyl]phenoxy}malonic acid (19) was prepared from racemic *N*-Boc-homotyrosine[35] by procedures analogous to those described for the transformation of **2** to **14** (Scheme 3). Preparative reverse phase HPLC, using the same conditions as reported for the purification of **20**, afforded **19** as a white solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.07 (d, J = 8 Hz, 1 H), 7.76 (t, J = 6 Hz, 1 H), 7.06 (d, J = 9 Hz, 2 H), 6.80 (d, J = 9 Hz, 2 H), 4.61 (s, 1 H), 4.16−4.15 (m, 1 H), 3.06−2.98 (m, 2 H), 2.49−2.37 (m, 6 H), 1.92−1.82 (m, 1 H), 1.77−1.66 (m, 1 H), 1.40−1.35 (m, 2 H), 1.27−1.20 (m, 4 H), 0.84 (t, J = 7 Hz, 3 H); MS (ESI−) for $C_{23}H_{31}NO_9$ − CO_2 *m/z* 421.2; HPLC retention time = 27 min.

2-{4-[2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]-2-fluorophenoxy}malonic acid (20) was prepared from racemic *N*-Boc-3-fluorotyrosine by procedures analogous to those described for the transformation of **2** to **14** (Scheme 3). Following preparative reverse phase HPLC, **20** was obtained as a hygroscopic white solid after lyophilization: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.08 (d, J = 8 Hz, 1 H), 7.82 (br t, 1 H), 7.07 (d, J = 12 Hz, 1 H), 6.96−6.89 (m, 2 H), 5.33 (s, 1 H), 4.39−4.32 (m, 1 H), 3.06−2.94 (m, 2 H), 2.90−2.84 (m, 1 H), 2.99−2.63 (m, 1 H), 2.35−2.21 (m, 4 H), 1.46−1.11 (m, 6 H), 0.82 (t, J = 7 Hz, 3 H); MS (FAB) *m/z* 471 (MH^+), 471, 193, 171, 167, 153, 135, 133, 121, 103, 89; HRMS (FAB) calcd for $C_{21}H_{27}FN_2O_9$ + H 471.1779, found 471.1797. Anal. ($C_{21}H_{27}FN_2O_9$·3.0H_2O) C, N; H: calcd, 6.34; found, 5.45.

Preparative RP HPLC Conditions.

Column: Rainin Dynamax C8, 8 μm particle size, 60 Å pore size, 21.4 × 250 mm.

Solvent: isocratic with 20% MeCN/H_2O/0.1% TFA, 6 mL/min

. λ = 210 nm.

Injection volume = 2000 μL.

Sample prepared by dissolving crude material into 2 mL of mobile phase.

t_R of major peak = 134 min.

2-{4-[2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]-2,3,5,6-tetrafluorophenoxy}malonic acid (21) was prepared from *N*-Boc-L-2,3,5,6-tetrafluorotyrosine[33] by procedures analogous to those described for the transformation of **2** to **14** (Scheme 3). **21** was obtained as an off-white solid: mp 122 °C dec; $[\alpha]^{25}_D = -6°$(*c* 0.54, ethanol); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.15 (br d, J = 8 Hz, 1 H), 7.91 (br t, 1 H), 5.32 (s, 1 H), 4.47 (q, J = 8 Hz, 1 H), 3.07−2.97 (m, 2 H), 2.92−2.82 (m, 2 H), 2.33−2.30 (m, 4 H), 1.27−1.07 (m, 6 H), 0.82 (t, J = 7 Hz, 3 H); MS (FAB) *m/z* 525 (MH^+), 525, 177,

133, 101, 88, 86, 74, 55, 43, 23; HRMS (FAB) calcd for $C_{21}H_{24}F_4N_2O_9$ + H 525.1496, found 525.1495.

Benzyl 4-{[(1*S*)-2-(*tert*-Butoxy)-1-(4-hydroxybenzyl)-2-oxoethyl]amino}-4-oxobutanoate (26a). To a stirring solution of H-Tyr-OBu^t (**25a)** (3.0 g, 12.64 mmol) in CH_2Cl_2 (150 mL) at 0 °C was added EDC (2.42 g, 12.64 mmol) and monobenzyl succinate (2.62 g, 12.64 mmol). The mixture was stirred for 16 h, the solution being allowed to warm to ambient temperature. The resulting solution was washed with 10% HCl/H_2O (150 mL), and the organic phase was dried over Na_2SO_4 and evaporated in vacuo. The residue was purified via flash column chromatography (2:1 hexane/EtOAc) to afford 5.06 g (94%): ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9 H), 2.50 (m, 2 H), 2.67 (m, 2 H), 2.99 (m, 2 H), 4.68 (m, 1 H), 5.11 (s, 2 H), 5.54 (brs, 1 H), 6.12 (d, 1 H), 6.71 (d, 2 H), 6.98 (d, 2 H), 7.33 (m, 5 H).

Dibenzyl 2-{4-[(2*S*)-2-{[4-(Benzyloxy)-4-oxobutanoyl]amino}-3-(*tert*-butoxy)-3-oxopropyl]phenoxy}malonate (27a). To a stirring solution of **26a** (3.55 g, 8.31 mmol) in acetone (175 mL) at ambient temperature was added K_2CO_3 (2.29 g, 16.62 mmol). Dibenzyl bromomalonate (3.31 g, 9.14 mmol) was added, and the mixture was stirred at ambient temperature overnight. The solvent was removed in vacuo, and the residue was suspended between EtOAc/H_2O (100 mL each). The layers were shaken, and the organic layer was separated and dried over Na_2SO_4, and the solvent was removed. The residue was purified via flash chromatography to afford 1.72 g (29%) title compound as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9 H), 2.49 (m, 2 H), 2.68 (m, 2 H), 3.00 (d, 2 H), 4.66 (m, 1 H), 5.11 (s, 2 H), 5.23 (s, 4 H), 5.24 (s, 1 H), 6.05 (d, 1 H), 6.82 (d, 2 H), 7.03 (d, 2 H), 7.33 (m, 15 H).

(2*S*)-3-(4-{2-(Benzyloxy)-1-[(benzyloxy)carbonyl]-2-oxoethoxy}phenyl)-2-{[4-(benzyloxy)-4-oxobutanoyl]amino}propanoic Acid (28a). To a stirring solution of **27a** (1.56 g, 2.20 mmol) in CH_2Cl_2 (40 mL) was added trifluoroacetic acid (100 mL). The resulting solution was stirred for 2 h and solvent removed in vacuo to afford 1.42 g (quant) of **28a** as a slightly pink oil: ^{1}H NMR ($CDCl_3$) δ 2.52 (m, 2 H), 2.67 (t, 2 H), 3.07 (ddd, 2 H), 4.81 (q, 1 H), 5.10 (s, 2 H), 5.20 (s, 4 H), 5.25 (s, 1 H), 6.43 (d, 1 H), 6.83 (d, 2 H), 7.40 (d, 2 H), 7.31 (M, 15 H), 7.80 (brs, 1 H).

General Procedure for Coupling of Amines with 28a To Afford Amides 29a. To a stirring solution of **28a** in CH_2Cl_2 (0.035 M) at 0 °C was added EDC (1 equiv), followed by the requisite amine. The mixture was stirred for 16 h, allowing the solution to warm to ambient temperature. The mixture was diluted with CH_2Cl_2 (50 mL) and then washed with 10% HCl/H_2O and then saturated $NaHCO_3$. The solvent was dried over Na_2SO_4 and removed in vacuo. Purification by SiO_2 flash column chromatography (eluant 1:1 EtOAc/hexane) afforded the desired amide.

General Procedure for Hydrogenation of Benzyl Esters 29a to Acids 30. To a stirring solution of the requisite ester in MeOH (0.03 M) was added 10% Pd−C (10% w/w). The resulting mixture was hydrogenated at atmospheric pressure for 3 h and filtered through Celite. The solvent was removed in vacuo to afford the desired triacids.

Methyl 4-{[(1*S*)-2-(Benzyloxy)-1-(4-hydroxybenzyl)-2-oxoethyl]amino}-4-oxobutanoate (26b). To a mixture of L-tyrosine benzyl ester *p*-toluenesulfonate salt (**25b**)(5.0 g, 11.3 mmol) and triethylamine in CH_2Cl_2 (25 mL) at 0 °C were added EDC (2.2 g, 11.3 mmol) and monomethyl succinate (1.5 g, 11.3 mmol). The mixture was warmed to room temperature and stirred overnight. The mixture was diluted with EtOAc (150 mL) and washed with 1 M HCl (50 mL), sat. $NaHCO_3$ (50 mL), and sat. NaCl (50 mL). The organic phase was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (SiO_2, 60% EtOAc/hexane) to provide 3.4 g (78%) of **26b** as a colorless oil that slowly solidified to a white solid: ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 5 H), 6.84 (d, 2 H), 6.65 (d, 2 H), 6.15 (d, 1 H), 5.62 (br s, 1 H), 5.14 (q, 2 H), 4.87 (m, 1 H), 3.67 (s, 3 H), 3.01 (m, 2 H), 2.64 (m, 2 H), 2.47 (m, 2 H).

Diethyl 2-(4-{(2*S*)-3-(Benzyloxy)-2-[(4-methoxy-4-oxobutanoyl)amino]-3-oxopropyl}phenoxy)malonate (27b). To a mixture of **26b** (2.64 g, 6.85 mmol) and K_2CO_3 (1.9 mmol, 13.7 mmol) in acetone (20 mL) was added diethyl chloromalonate (2.2 mL, 13.7 mmol). The mixture was stirred vigorously for 18 h. The mixture was partitioned between EtOAc and H_2O. The organic phase was washed with sat. $NaHCO_3$ and sat. NaCl. After drying ($MgSO_4$), the solvent was removed under reduced pressure. The residue was purified by flash chromatography (50% EtOAc/hexane) to provide 2.9 g of **27b** as a colorless oil (contaminated with approximately 10% of the corresponding α-chloromalonate): ^{1}H NMR ($CDCl_3$) δ 7.37 (m, 3 H), 7.29 (m, 2 H), 6.90 (d, 2 H), 6.80 (d, 2 H), 6.06 (d, 1 H), 5.12 (m, 3 H), 4.86 (q, 1 H), 4.31 (q, 4 H), 3.67 (s, 3 H), 3.05 (m, 2 H), 2.62 (m, 2 H), 2.48 (t, 2 H), 1.30 (t, 6 H).

(2*S*)-3-{4-[2-Ethoxy-1-(ethoxycarbonyl)-2-oxoethoxy]-phenyl}-2-[(4-methoxy-4-oxobutanoyl)amino]propanoic Acid (28b). A Parr flask was charged with **27b** (150 mg, 0.28 mmol), 10% Pd/C (25 mg), and abs. EtOH (25 mL), and the mixture was hydrogenated (35 psi) for 45 min. The mixture was filtered through Celite and concentrated to provide 110 mg (87%) of **28b** as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 7.11 (d, 2 H), 6.88 (d, 2 H), 6.23 (d, 1 H), 5.17 (s, 1 H), 4.79 (q, 1 H), 4.30 (q, 4 H), 3.67 (s, 3 H), 3.15 (ab q, 1 H), 3.06 (ab q, 1 H), 2.63 (t, 2 H), 2.28 (t, 2 H), 1.30 (t, 6 H).

General Procedure for the Preparation of 30 from 28b (Scheme 4). To a mixture of **28b** (1 equiv) in CH_2Cl_2 (0.2 M) at 0 °C was added EDC (1 equiv) followed by the requisite amine. The reaction was warmed to room temperature and stirred for 18 h. The mixture was diluted with EtOAc and washed with 1 M HCl, sat. $NaHCO_3$, and sat. NaCl. The organic phase was dried ($MgSO_4$) and concentrated under reduced pressure to give the crude ester **29b**. The residue was dissolved in THF (3 mL), and a solution of LiOH·H_2O (6−8 equiv) in H_2O (1 mL) was added. The mixture was stirred for 2−4 h. The mixture was acidified with 1M HCl and extracted with EtOAc (3×). The combined organic phase was washed with sat. NaCl and dried ($MgSO_4$). The solvent was removed in vacuo to provide **30**.

Dibenzyl 2-{4-[(2*S*)-2-({(2*S*)-4-(Benzyloxy)-2-[(*tert*-butoxycarbonyl)amino]-4-oxobutanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonate (31). To a stirring solution of **12a** (0.25 g, 0.44 mmol) and triethylamine (0.044 g, 0.44 mmol) was added Boc-Asp(OBn)OSu (0.185 g, 0.44 mmol) at 0 °C. The resulting solution was stirred for 16 h, and the mixture was allowed to warm to ambient temperature. The resulting solution was diluted with CH_2Cl_2 (50 mL) and washed with 10% aqueous HCl. The organic layer was dried over $MgSO_4$ and solvent removed to afford 0.33 g (87%) of **31** as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H), 1.15−1.43 (m, 15 H), 2.76 (m, 1 H), 2.93 (m, 2 H), 3.09 (m, 3 H), 4.41 (m, 1 H), 4.48 (m, 1 H), 5.10 (s, 2 H), 5.23 (s, 4 H), 5.29 (s, 1 H), 5.44 (d, 1 H), 5.91 (m, 1 H), 6.89 (d, 1 H), 6.85 (d, 2 H), 7.10 (d, 2 H), 7.30 (m, 15 H).

2-{4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-carboxypropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-phenoxy}malonic Acid (32). To a solution of **31** (0.317 g, 0.38 mmol) in MeOH (15 mL) at ambient temperature was added 10% Pd−C (50 mg), and the resulting mixture was hydrogenated at atmospheric pressure for 2 h. The mixture was filtered through Celite and the solvent removed to afford 0.208 g (97%) of **31** as a white solid: ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.12−1.40 (m, 15 H), 2.30−2.52 (m, 2 H), 2.70−3.06 (m, 4 H), 4.16 (m, 1 H), 4.32 (m, 1 H), 5.20 (s, 1 H), 6.76 (d, 2 H), 7.60 (m, 2 H), 7.12 (d, 1 H), 7.69 (d, 1 H), 7.78 (t, 1 H) (3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 568 (MH^+, 9), 512 (31), 468 (23), 88 (99), 88 (25), 86 (20), 57 (95), 43 (21), 41 (28), 29 (26), 23 (27). Anal. ($C_{26}H_{37}N_3O_{11}$·1.48H_2O) C, H, N.

4-{[(1*S*)-3-(Benzyloxy)-1-({[(1*S*)-1-(4-{2-(benzyloxy)-1-[(benzyloxy)carbonyl]-2-oxoethoxy}benzyl)-2-oxo-2-(pentylamino)ethyl]amino}carbonyl)-3-oxopropyl]amino}-4-oxobutanoic Acid (33). To a solution of **31** (0.263 g, 0.31 mmol) in HOAc (5 mL) was added 1.5 M HCl/HOAc (5 mL), and the resulting solution was allowed to stand for 2 h. The

solvent was removed in vacuo to afford 0.24 g (quant) of the amine hydrochloride as a white solid. To a stirring solution of this crude amine (0.10 g, 0.13 mmol) and triethylamine (0.028 g, 0.28 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added succinic anhydride (0.015 g, 0.13 mmol). The resulting solution was stirred for 16 h, the solution being allowed to warm to ambient temperature. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with 10% aqueous HCl. The organic layer was dried over $MgSO_4$ and concentrated to afford 0.076 g (70%) of **33** as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H), 1.23 (m, 4 H), 1.40 (m, 2 H), 2.37 (m, 2 H), 2.68 (m, 2 H), 3.14 (m, 6 H), 4.57 (m, 1 H), 4.72 (m, 1 H), 5.01 (dd, 2 H), 5.16 (s, 4 H), 5.21 (s, 1 H), 6.36 (t, 1 H), 6.80 (m, 2 H), 7.05 (d, 2 H), 7.25 (m, 17 H) (CO_2H absent).

2-{4-[(2*S*)-2-({(2*S*)-3-Carboxy-2-[(3-carboxypropanoyl)-amino]propanoyl}amino)-3-oxo-3-(pentylamino)propyl]-phenoxy}malonic Acid (34). To a stirring solution of **33** (0.07 g, 0.08 mmol) in MeOH (5 mL) was added 10% Pd−C (10 mg). The resulting mixture was hydrogenated at atmospheric pressure for 2 h and filtered through Celite. The solvent was removed to afford 0.044 g (94%) of **34** as a white amorphous solid: ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.20 (m, 6 H), 2.33 (m, 3 H), 2.43 (m, 3 H), 2.72 (m, 1 H), 2.96 (m, 3 H), 4.27 (m, 1 H), 4.42 (m, 1 H), 5.2 (s, 1 H), 6.74 (d, 2 H), 7.06 (d, 2 H), 7.60 (t, 1 H), 7.70 (d, 1 H), 8.30 (d, 1 H) (4-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 568 (MH^+, 99), 582 (26), 569 (32), 568 (99), 238 (40), 177 (42), 102 (37), 88 (56), 88 (53), 39 (24), 23 (26). Anal. ($C_{25}H_{33}N_3O_{12}$·1.1H_2O) C, H, N.

2-{4-[(2*S*)-2-{[(2*S*)-3-Carboxy-2-(hexanoylamino)pro-panoyl]amino}-3-oxo-3-(pentylamino)propyl]phenoxy}-malonic Acid (35). To a stirring solution of the amine hydrochloride derived from deprotection of **31** (as described for preparation of **33**) (0.25 g, 0.32 mmol) in CH_2Cl_2 (8 mL) at 0 °C was added hexanoyl chloride (0.043 g, 0.32 mmol). The resulting solution was stirred for 16 h, the mixture being allowed to warm to ambient temperature. The solution was diluted with CH_2Cl_2 (20 mL) and washed with 10% HCl/H_2O (3 × 50 mL). The organic layer was dried over Na_2SO_4 and concentrated to afford 0.164 g (61%) of the amide as a white solid. To a stirring solution of crude amide (0.175 g, 0.22 mmol) in MeOH (15 mL) was added 10% Pd−C (25 mg). The mixture was hydrogenated at atmospheric pressure for 3 h and filtered through Celite. The solvent was removed to afford 0.113 g (87%) **35** as a white solid: ^{1}H NMR (DMSO) δ 0.82 (m, 6 H), 1.22 (m, 10 H), 1.44 (m, 2 H), 2.04 (t, 2 H), 2.38 (m, 1 H), 2.62 (m, 1 H), 2.63−3.10 (m, 4 H), 4.32 (m, 1 H), 4.51 (m, 1 H), 5.19 (s, 1 H), 6.75 (d, 2 H), 7.04 (d, 2 H), 7.74 (m, 2 H), 8.10 (d, 1 H) (3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 566 (MH^+, 37), 588 (24), 566 (37), 177 (38), 99 (26), 88 (56), 88 (88), 71 (29), 43 (99), 39 (54), 23 (89). Anal. ($C_{27}H_{39}N_3O_{10}$·0.78H_2O) C, H, N.

Diethyl 2-{4-[(2*S*)-2-({(2*S*)-3-(4-Benzoylphenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonate (36). To a mixture of L-Boc-Bpa-OH (0.93 g, 2.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added EDC (0.48 g, 2.5 mmol) and HOBT (0.34 g, 2.5 mmol). After stirring for a few minutes, **12b** (1.12 g, 2.5 mmol) and triethylamine (0.39 mL, 2.8 mmol) were added. The mixture was warmed to room temperature and stirred overnight. The mixture was partitioned between 1 M HCl and EtOAc. The organic phase was washed with sat. $NaHCO_3$ and sat. NaCl and dried ($MgSO_4$). The solvent was removed under reduced pressure, and the residue was purified by flash chromatography (85 g of SiO_2, 25% EtOAc/hexane) to provide 1.0 g (58%) of **36** as a white solid: ^{1}H NMR (DMSO-d_6) δ 8.00 (d, 1 H), 7.91 (t, 1 H), 7.60−7.70 (m, 5 H), 7.54 (t, 2 H), 7.13 (d, 2 H), 6.97 (d, 1 H), 6.83 (d, 2 H), 5.58 (s, 1 H), 4.40 (m, 1 H), 4.16 (m, 5 H), 2.80−3.00 (m, 4 H), 2.75 (m, 2 H), 1.28 (m, 10 H), 1.68 (m, 11 H), 0.81 (t, 3 H).

2-{4-[(2*S*)-2-({(2*S*)-3-(4-Benzoylphenyl)-2-[(*tert*-butoxycarbonyl)amino]propanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (37) was prepared by direct LiOH saponification of **36** with isolation as described for **38**, affording **37**: ^{1}H NMR (DMSO-d_6) δ 8.00 (d, 1 H), 7.88 (t, 1 H), 7.66 (m, 5 H), 7.53 (t, 2 H), 7.12 (d, 2 H), 7.00 (d, 1 H), 6.80 (d, 2 H), 5.23 (s, 1 H), 4.40 (m, 1 H), 4.30 (m, 1 H), 2.70−3.05 (m, 6 H), 1.10−1.30 (m, 15 H), 0.80 (t, 3 H); MS (FAB) *m*/*z* (rel intensity) 704 (MH^+, 6), 648 (30), 238 (23), 224 (49), 194 (30), 136 (20), 105 (33), 88 (99), 57 (88), 43 (24), 41 (22); HRMS (FAB) calcd for $C_{38}H_{45}N_3O_{10}$ + H 704.3183, found 704.3171. Anal. ($C_{38}H_{45}N_3O_{10}$·0.80H_2O) C, H, N.

2-{4-[(2*S*)-2-({(2*S*)-3-(4-Benzoylphenyl)-2-[(3-carboxypropanoyl)amino]propanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic Acid (38). Compound **36** (0.34 mmol) was dissolved in a 1 M solution of HCl in acetic acid (4 mL) and stirred at room temperature for 3 h. The solvent was removed under reduced pressure, and the residue was dissolved in a mixture of triethylamine (0.75 mmol) in CH_2Cl_2 (1.5 mL). The mixture was cooled to 0 °C, and succinic anhydride (0.34 mmol) was added. The mixture was warmed to room temperature and stirred overnight. The mixture was partitioned between EtOAc and 1 M HCl, and the organic phase was washed with sat. NaCl and dried ($MgSO_4$). After the solvent was removed, the residue was dissolved in THF (3 mL), and a solution of LiOH·H_2O (1−2 mmol) in H_2O (1 mL) was added. The mixture was stirred for 2−5 h. The mixture was neutralized with 1 M HCl and extracted with EtOAc (2×). The combined organic phases were washed with sat. NaCl and dried ($MgSO_4$). The solvent was removed in vacuo to provide **38** (169 mg, 70%) as an off-white solid: ^{1}H NMR (DMSO) δ 8.12 (d, 1 H), 8.05 (d, 1 H), 7.75 (t, 1 H), 7.70−7.75 (m, 7 H), 7.35 (d, 2 H), 7.11 (d, 2 H), 6.81 (d, 2 H), 5.25 (s, 1 H), 4.51 (m, 1 H), 4.35 (m, 1 H), 2.70−3.05 (m, 6 H), 2.32 (m, 4 H), 1.10−1.35 (m, 6 H), 0.80 (t, 3 H); MS (FAB) *m*/*z* (rel intensity) 704 (MH^+, 88), 705 (39), 704 (88), 353 (30), 238 (36), 224 (99), 194 (25), 136 (23), 107 (14), 105 (29), 88 (40); HRMS (FAB) calcd for $C_{37}H_{41}N_3O_{11}$ + H 704.2819, found 704.2804. Anal. ($C_{37}H_{41}N_3O_{11}$·0.63H_2O) C, H, N.

Methyl 4-{[(1*S*)-1-(4-hydroxybenzyl)-2-oxo-2-(pentyl-amino)ethyl]amino}-4-oxobutanoate (39) was prepared as described for **6** using (mono)methyl succinate: ^{1}H NMR ($CDCl_3$) 7.04 (d, J = 8 Hz, 2 H), 6.74 (d, J = 8 Hz, 2 H), 6.40 (bs, 1 H), 6.32 (d, J = 8 Hz, 1 H), 6.04 (bt, J = 8 Hz, 1 H), 4.55 (q, J = 7 Hz, 1 H), 3.66 (s, 3 H), 3.13 (q, J = 7 Hz, 2 H), 3.03, 2.92 (ABqd, J = 7, 14 Hz, 2 H), 2.5−2.8 (m, 2 H), 2.45 (m, 2 H), 1.1−1.4 (m, 6 H), 0.86 (t, J = 7 Hz, 3 H).

4-{[(1*S*)-1-{4-[(2-Carboxyacetyl)oxy]benzyl}-2-oxo-2-(pentylamino)ethyl]a mino}-4-oxobutanoic Acid (40). To a mixture of **6** (100 mg, 0.227 mmol) and benzyl malonyl chloride (72 mg, 0.34 mmol) in methylene chloride (1 mL) was added pyridine (40 μL). The mixture immediately went into solution. After 1 h, the solution was diluted with ethyl acetate and washed successively with dilute HCl, water, and sat. sodium bicarbonate. Drying of the organic phase and concentration left a yellow viscous oil (158 mg). NMR analysis showed some starting material left, so the reaction was repeated for 19 h. The two crude reaction products were combined and flash chromatographed (30 g, 80% ethyl acetate/hexane), providing the desired ester (90 mg, 32%) as the highest R_f material: ^{1}H NMR ($CDCl_3$) 7.35 (m, 5H), 7.20 (d, 2H, J = 8 Hz), 6.98 (d, 2H, J = 8 Hz), 6.12 (d, 1H, J = 8 Hz), 5.88 (bt, 1H, J = 6 Hz), 5.23 (s, 2H), 5.11 (s, 2H), 4.57 (m, 1H), 3.64 (s, 2H), 3.12 (m, 3H), 2.98 (dd, 1H, J = 8, 14 Hz), 2.6−2.9 (m, 2H), 2.45 (m, 2H), 1.1−1.4 (m, 6H), 0.86 (t, 3H, J = 7 Hz).

A mixture of the ester from above (84 mg, 0.14 mmol) and 10% Pd/C (20 mg) in methanol (7 mL) was hydrogenated at 1 atm for 1 h. Concentration in vacuo left a colorless glass (58 mg, 95%). NMR analysis indicated the desired product contaminated with 5−10% of the corresponding free phenol: ^{1}H NMR (DMSO) 8.09 (d, 1H, J = 7 Hz), 7.81 (bt, 1H, J = 6 Hz), 7.23 (d, 2H, J = 8 Hz), 6.98 (d, 2H, J = 8 Hz), 4.4 (m, 1H), 3.61 (s, 2H), 2.95 (m, 3H), 2.7 (m, 1H), 2.3 (m, 4H), 1.1−1.4 (m, 6H), 0.82 (t, 3H, J = 7 Hz); HRMS (FAB) calcd for $C_{21}H_{28}N_2O_8$ + H 437.1924, found 437.1924.

4-{[(1*S*)-1-[4-(Carboxymethoxy)benzyl]-2-oxo-2-(pentyl-amino)ethyl]amino}-4-oxobutanoic Acid (41). A mixture

of **6** (300 mg, 0.681 mmol), potassium bicarbonate (136 mg, 1.36 mmol), and benzyl bromoacetate (216 μL, 1.36 mmol) in acetone (5 mL) was stirred at 50−55 °C for 3 days. TLC analysis showed some starting material left, so another 1 equiv of bromide was added, and the mixture was stirred a further 24 h. Aqueous workup (ethyl acetate/water/brine) gave a solid and oil. Flash chromatography (60 g silica, 1/1 ethyl acetate: methylene chloride) gave a colorless oil (R_f = 0.45, 285 mg) that was impure product. This material was left standing in methylene chloride:hexane at 0 °C overnight, producing some crystals in an oil. After sonication, all of the material became a white solid. Filtration gave 166 mg of the ether diester (41%) after washing with hexane and drying (mp 102−103 °C): ^{1}H NMR ($CDCl_3$) 7.34 (m, 5H), 7.12 (d, 2H, J = 9 Hz), 6.82 (d, 2H, J = 9 Hz), 6.10 (d, 1H, J = 8 Hz), 5.81 (bt, 1H, J = 7 Hz), 5.23 (s, 2H), 5.12 and 5.08 (Abq, 2H, J = 12 Hz), 4.63 (s, 2H), 4.52 (m, 1H), 3.1 (m, 2H), 2.90 (dd, 1H, J = 8, 14 Hz), 2.6−2.85 (m, 2H), 2.45 (m, 2H), 1.1−1.4 (m, 6H), 0.86 (t, 3H, J = 7 Hz).

A mixture of the diester from above (100 mg, 0.170 mmol) and 10% Pd/C (20 mg) in methanol (10 mL) was hydrogenated at 1 atm for 1.5 h. The mixture was filtered through Celite and concentrated in vacuo to a colorless glass (69 mg, approximately 100%). Sonication in methylene chloride and evaporation of the solvent left a white powdery solid (mp 106−109 °C): ^{1}H NMR (MeOD) 7.81 (t, 1H, J = 6 Hz), 7.15 (d, 2H, J = 9 Hz), 6.84 (d, 2H, J = 9 Hz), 4.60 (s, 2H), 4.49 (dd, 1H, J = 6.3, 8.5 Hz), 3.1 (m, 3H), 2.81 (dd, 1H, J = 8.6, 14 Hz), 2.3−2.7 (m, 4H), 1.1−1.4 (m, 6H), 0.88 (t, 3H, J = 7 Hz); ^{13}C NMR (MeOD) 175.0, 173.1, 172.0, 171.4, 156.9, 130.1, 129.9, 114.2, 64.6, 55.1, 39.2, 39.1, 36.7, 30.0, 28.7, 28.5, 22.0, 12.9; MS (ESI−) for $C_{20}H_{28}N_2O_7$ m/z 407.0 $(M - H)^-$; MS (FAB) m/z (rel intensity) 409 (MH^+, 99), 432 (17), 431 (40), 410 (45), 409 (99), 291 (16), 194 (73), 89 (22), 88 (97), 86 (16), 43 (20); HRMS (FAB) calcd for $C_{20}H_{28}N_2O_7$ + H 409.1974, found 409.1985. Anal. ($C_{20}H_{28}N_2O_7$·0.3H_2O) C, H, N.

4-{[(1S)-1-{4-[(Methylsulfonyl)oxy]benzyl}-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoic Acid (42). A magnetically stirred suspension of **6** (320 mg, 0.73 mmol) in dichloromethane (5 mL) was cooled to 0 °C and treated dropwise with triethylamine (0.5 mL) and mesyl chloride (70 uL, 1.2 equiv) under N_2 atmosphere. The reaction mixture was allowed to warm to ambient temperature and after 1 h. TLC showed complete conversion. Dichloromethane (30 mL) was added and the organic layer was washed with aqueous HCl (10%, 25 mL) and dried over $MgSO_4$. Filtration and removal of the solvent in vacuo gave 0.40 g of an off-white solid. Recrystallization from acetonitrile gave a first crop of mesylate ester (0.24 g crystalline white solid). A second crop gave an additional 50 mg. The total yield amounted to 0.29 g (77%): mp 167.1−168.3 °C; ^{1}H NMR ($CDCl_3$) δ 0.86 (t, J = 7.1, 3H), 1.15−1.30 (m, 4H), 1.35−1.43 (m, 2H), 2.43−2.50 (m, 2H), 2.61−2.79 (m, 2H), 3.07−3.17 (m, 4H), 3.12 (s, 3H), 4.65 (dt, J_1 = 8.1, J_2 = 6.9, 1H), 5.10 (s, 2H), 6.23 (t, J = 5.6, 1H), 6.38 (d, J = 8.2, 1H), 7.19 (d, J = 8.7, 2H), 7.25 (d, J = 8.7, 2H), 7.30−7.37 (m, 5H).

To a stirred solution of the above ester (233 mg, 449 μmol) in THF (6 mL) were added aqueous LiOH (2.5 M, 400 μL, 2.2 equiv) and methanol (1.5 mL). After 30 min the reaction mixture was taken up in sat. aq $NaHCO_3$ (30 mL) and washed with diethyl ether (25 mL). The aqueous layer was then acidified until pH 3 with 10% HCl and extracted with ethyl acetate (3 × 20 mL). The combined organic layers were dried (Na_2SO_4) and evaporated in vacuo, giving 194 mg of a white solid that was recrystallized from acetone. This afforded 128 mg of **42** (67%) as white yellow crystals: mp 165.0−166.2 °C; ^{1}H NMR (CD_3OD) δ 0.91 (t, J = 7.0, 3H), 1.21−1.37 (m, 4H), 1.41−1.48 (m, 2H), 2.37−2.64 (m, 4H), 2.92 (dd, $J_{1,2}$ = 8.8, 1H), 3.10−3.22 (m, 3H), 3.20 (s, 3H), 4.58 (dd, J_1 = 8.7, J_2 = 8.6, 1H), 7.25 (d, J = 8.6, 2H), 7.35 (d, J = 8.6, 2H); ^{13}C NMR (CD_3-OD) δ 14.78, 23.79, 30.36, 30.52, 31.75, 37.82, 38.62, 40.88, 41.01, 56.50, 123.59, 132.28, 138.61, 150.20, 173.50, 174.97, 176.75; MS (ionspray, $[M - H]^-$) m/z 427.2. Anal. ($C_{19}H_{28}N_2O_7S$) C, H, N.

Dimethyl (*Z*)-2-{4-[(2*S*)-2-[(4-Methoxy-4-oxobutanoyl)-amino]-3-oxo-3-(pentylamino)propyl]phenoxy}-2-butenedioate (44). To a mixture of **39** (0.37 g, 1.0 mmol) and triethylamine (0.17 mmol, 1.2 mmol) in THF (5 mL) was added dimethyl acetylenedicarboxylate (0.25 mL, 2.0 mmol), and the mixture was heated at 50 °C overnight. The mixture was diluted with Et_2O and washed with 1 M HCl and sat. NaCl. The organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography (SiO_2, 90% EtOAc/hexane) to yield 0.41 g (79%) of **44** as a 1:1 mixture of isomers: ^{1}H NMR ($CDCl_3$) δ (all peaks overlap except aromatic and olefin) nonoverlapping peaks, 7.26 (d, 2 H), 7.15 (d, 2 H), 7.04 (d, 2 H), 6.89 (d, 2 H) 6.58 (s,1 H, fumarate), 5.12 (s, 1 H, maleate), 3.91 (s, 3 H, methyl ester); overlapping peaks, 6.16 (t, 1 H, NH), 5.94 (m, 1 H, NH), 4.56 (m, 1 H), 3.73, 3.70, 3.67 (s, methyl ester), 2.92−3.20 (m, 4 H), 2.55−3.80 (m, 2 H), 2.43 (m, 2 H), 1.38 (m, 2 H), 1.25 (m, 4 H), 0.87 (t, 3 H).

(*Z*)-2-{4-[(2*S*)-2-[(4-Hydroxy-4-oxobutanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}-2-butenedioic Acid (45). To a mixture of **44** (100 mg, 0.20 mmol) in MeOH (5 mL) was added a solution of LiOH·H_2O (50 mg, 1.2 mmol) in H_2O (1.5 mL), and the mixture was stirred at room temperature overnight. The reaction mixture was concentrated in vacuo, and the residue was dissolved in H_2O. After cooling to 0 °C, 1 M HCl was added until pH = 1. The solid that slowly precipitated over several hours at 0 °C was collected and dried to provide 30 mg (32%) of **45** (6:1 mixture of isomers) as a slightly yellow solid: ^{1}H NMR (MeOH) δ 7.18 (d, 2 H, major isomer), 6.86 (d, 2 H, major isomer), 6.55 (s, 1 H, major isomer), 5.10 (s, 1 H, minor isomer), 4.49 (m, 1 H), 3.10 (m, 3 H), 2.87 (m, 1 H), 2.39−2.54 (m, 4 H), 1.27−1.45 (m, 6 H), 0.90 (t, 3 H); the major isomer was assigned as *E* on the basis of the relative chemical shifts of olefinic protons; HRMS (FAB) calcd for $C_{22}H_{28}N_2O_9$ + H 465.1873, found 465.1856. Anal. ($C_{22}H_{28}N_2O_9$·1.28H_2O) C, H, N.

2-{4-[(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}succinic Acid (46). To a suspension of **6** (50 mg, 0.11 mmol) and triethylamine (18 mL, 0.13 mmol) in THF (0.3 mL) was added a solution of dibenzyl acetylenedicarboxylate (35 mg, 0.12 mmol) in THF (0.2 mL). The reaction mixture was heated at 50 °C for 20 h. The mixture was diluted with Et_2O and washed with 1 M HCl and sat. NaCl. The organic phase was dried (Na_2SO_4), and the solvent was removed in vacuo. The residue was purified by flash chromatography (9 g SiO_2, 60% EtOAc/hexane) to provide 48 mg (59%) of **43** as a 1:1 mixture of isomers: ^{1}H NMR ($CDCl_3$) δ (all peaks overlap except aromatic and olefin) nonoverlapping, 7.21 (d, 2 H), 7.10 (d, 2 H), 7.02 (d, 2 H), 6.86 (d, 2 H), 6.62 (s, 1 H, fumarate), 5.30 (s, 1 H, maleate); overlapping, 7.30−7.40 (m, 5 H, Ph benzyl ester), 6.11 (m, 1 H, NH), 5.90 (m, 1 H, NH), 5.06−5.21 (complex, CH_2 benzyl esters), 4.55 (m, 1 H), 3.10 (m, 3 H), 2.90−3.00 (dd, 1 H), 2.60−2.85 (m, 2 H), 2.43 (m, 2 H), 1.10−1.40 (m, 6 H), 0.84 (m, 3 H); MS (ESI+) for $C_{43}H_{46}N_2O_9$ m/z 735 $(M + H)^+$, 757 (M + Na).

A mixture of **43** (48 mg) and 10% Pd/C (5 mg) in MeOH (2 mL) was stirred under a hydrogen atmosphere (balloon) for 1 h. The mixture was filtered through Celite and concentrated to provide 27 mg (90%) of **46** as a glass: ^{1}H NMR (DMSO) 8.02 (d, 1 H), 7.78 (m, 1 H), 7.08 (d, 2 H), 6.76 (d, 2 H), 4.92 (m, 1 H), 4.32 (m, 1 H), 2.6−3.0 (m, 6 H), 2.25−2.35 (m, 4 H), 1.1−1.4 (m, 6 H), 0.83 (t, 3 H); MS (FAB) m/z (rel intensity) 467 (MH^+, 71), 489 (22), 468 (17), 467 (71), 349 (11), 252 (38), 136 (13), 107 (11), 88 (99), 86 (17), 43 (20); HRMS (FAB) calcd for $C_{22}H_{30}N_2O_9$ + H 467.2029, found 467.2040.

***tert*-Butyl (1*S*)-1-(4-Formylbenzyl)-2-oxo-2-(pentylamino)ethylcarbamate (47).** To a stirring solution of **3** (5.0 g, 14.27 mmol) and pyridine (2.58 g, 71.38 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added trifluoromethanesulfonic anhydride (4.42 g, 15.70 mmol) dropwise over 15 min. After addition, the mixture was stirred for 1 h at 0 °C and diluted with CH_2Cl_2 (50 mL). The organics were washed with 10% HCl/H_2O (2 × 50 mL), separated, and dried over Na_2SO_4. The solvent was filtered through a pad of SiO_2 (50 g) and washed with CH_2Cl_2

(75 mL). The solvent was removed to afford 2.54 g (37%) of the triflate as a yellow solid: ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3 H), 1.18−1.50 (m, 6 H), 1.40 (s, 9 H), 3.09 (m, 4 H), 4.27 (m, 1 H), 5.07 (m, 1 H), 5.90 (t, 1 H), 7.19 (d, 2 H), 7.28 (d, 2 H).

To the stirring mixture of crude triflate from above (0.60 g, 1.24 mmol), LiCl (0.158 g, 3.73 mmol), and vinyltributyltin (0.591 g, 1.86 mmol) in DMF (10 mL) at ambient temperature was added dichlorobis(triphenylphosphine)palladium(II) (0.087 g, 0.12 mmol). The mixture was heated to 90 °C and stirred for 16 h. The resulting black mixture was cooled to ambient temperature, poured into ice/H_2O, and extracted with EtOAc (3 × 75 mL). The organic layers were combined and dried over Na_2SO_4, and the solvent was removed. The residue was purified via SiO_2 flash column chromatography (eluant 2:1 hexane/EtOAc) to afford 0.365 g (82%) of the aryl vinyl compound as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H), 1.13−1.40 (m, 6 H), 1.36 (s, 9 H), 3.06 (m, 4 H), 4.23 (m, 1 H), 5.06 (brs, 1 H), 5.22 (d, 1 H), 5.68 (brs, 1 H), 5.70 (d, 1 H), 6.67 (dd, 1 H), 7.15 (d, 2 H), 7.33 (d, 2 H).

O_3(g) was bubbled through a stirring solution of the vinyl compound from above (0.30 g, 0.83 mmol) in CH_2Cl_2 (50 mL) at −78 °C until the blue endpoint was observed. The reaction mixture was capped with a septum and stirred an additional 1.5 h at −78 °C. Dimethyl sulfide (0.78 g, 0.12 mmol) was added at −78 °C and the mixture was allowed to warm to ambient temperature (over 1 h). The solvent was removed, and the residue was taken up in Et_2O (50 mL) and washed with H_2O (2 × 50 mL). The organic layer was dried over Na_2SO_4, the solvent removed in vacuo, and the residue purified via SiO_2 flash column chromatography (eluant 2:1 hexane/EtOAc) to afford 0.172 g (57%) **47** as a white solid: ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3 H), 1.15−1.36 (m, 6 H), 1.39 (s, 9 H), 3.14 (m, 4 H), 4.30 (m, 1 H), 5.05 (brs, 1 H), 5.08 (brs, 1 H), 7.38 (d, 2 H), 7.81 (d, 2 H), 10.00 (s, 1 H).

Dibenzyl 2-{4-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]benzylidene}malonate (48). Compound **47** (0.50 g, 1.38 mmol), dibenzyl malonate (0.47 g, 1.65 mmol), piperidine (0.023 g, 0.27 mmol), and HOAc (3 drops) in benzene (15 mL) were heated to reflux for 2 h. The mixture was cooled to ambient temperature, diluted with EtOAc (50 mL), and washed with 10% HCl/H_2O (2 × 50 mL). The organic layer was dried over Na_2SO_4, the solvent removed, and the residue purified via SiO_2 flash column chromatography (eluant 2:1 hexane/EtOAc) to afford 0.060 g (70%) of **48** as a slightly yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.84 (t, 3 H), 1.17−1.39 (m, 6 H), 1.40 (s, 9 H), 3.02 (m, 2 H), 3.13 (m, 2 H), 4.21 (m, 1 H), 5.00 (brs, 1 H), 5.28 (m, 4 H), 5.67 (brt, 1 H), 7.09 (d, 2 H), 7.32 (m, 12 H), 7.72 (s, 1 H).

4-{[(1*S*)-1-(4-{3-(Benzyloxy)-2-[(benzyloxy)carbonyl]-3-oxo-1-propenyl}benzyl)-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoic Acid (49). To a stirring solution of **48** (0.65 g, 1.03 mmol) in HOAc (10 mL) was added 1.5 M HCl/HOAc (10 mL). The mixture was allowed to stand at ambient temperature for 2 h and the solvent removed to afford 0.58 g (quantitative) of the amine hydrochloride as a slightly yellow amorphous solid: ^{1}H NMR (DMSO) δ 0.76 (t, 3 H), 1.03 (m, 2 H), 1.17 (m, 4 H), 2.89 (m, 1 H), 3.02 (m, 3 H), 3.94 (m, 1 H), 5.25 (s, 2 H), 5.29 (s, 2 H), 7.22 (d, 2 H), 7.36 (m, 12 H), 7.73 (s, 1 H), 8.34 (brs, 2 H), 8.41 (t, 1 H).

To a stirring solution of the amine hydrochloride from above (0.507 g, 0.89 mmol) in CH_2Cl_2 (15 mL) at 0 °C was added triethylamine (0.20 g, 1.97 mmol), followed by succinic anhydride (0.089 g, 0.89 mmol). The mixture was stirred for 16 h, being allowed to warm to ambient temperature. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with 10% HCl/H_2O (2 × 50 mL). The organic layer was dried over Na_2SO_4 and solvent removed to afford 0.557 (99%) of **49** as a yellow amorphous solid: ^{1}H NMR ($CDCl_3$) δ 0.83 (t, 3 H), 1.11−1.28 (m, 6 H), 2.47 (m, 2 H), 2.63 (m, 2 H), 3.03 (m, 4 H), 4.57 (q, 1 H), 5.25 (s, 2 H), 5.27 (m, 2 H), 5.93 (t, 1 H), 6.88 (d, 1 H), 7.09 (d, 2 H), 7.27 (m, 12 H), 7.70 (s, 1 H) (CO_2H absent). Anal.-($C_{36}H_{40}N_2O_8$) C, H, N.

2-{4-[(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]benzylidene}malonic Acid (50). To a stirring solution of **49** (0.15 g, 0.23 mmol) in THF/MeOH (3:1 v/v, 5 mL) at ambient temperature was added LiOH/H_2O (2.5 M, 0.52 mL, 1.38 mmol). The mixture was stirred for 3 h and then acidified to pH ∼ 4 with 10% HCl/H_2O. The aqueous layer was extracted with EtOAc (2 × 50 mL), and the organic layers were combined and dried over Na_2SO_4. The solvent was removed to afford 0.062 g (61%) of **50** as a waxy solid: ^{1}H NMR (DMSO) δ 0.82 (m, 3 H), 1.13−1.17 (m, 6 H), 2.30 (m, 4 H), 2.78 (m, 1 H), 2.96 (m, 3 H), 7.20 (m, 1 H), 7.29 (m, 4 H), 7.45 (m, 1 H), 7.81 (m, 1 H), 8.10 (m, 1 H) (3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 449 (MH^+, 99), 481 (13), 450 (21), 449 (99), 363 (14), 177 (7), 133 (15), 118 (36), 88 (72), 86 (8), 43 (10).

2-{4-[(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]be nzyl}malonic Acid (51). To a stirring solution of **49** (0.15 g, 0.23 mmol) in MeOH (20 mL) was added 10% Pd−C (15 mg), and the mixture was hydrogenated at atmospheric pressure for 3 h. The solvent was filtered through Celite and removed in vacuo to afford 0.105 g (98%) **51** as an amorphous solid: ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.15−1.30 (m, 6 H), 2.31 (m, 4 H), 2.71 (m, 1 H), 2.96 (m, 5 H), 3.09 (s, 1 H), 4.35 (m, 1 H), 7.07 (s, 4 H), 7.77 (t, 1 H), 8.06 (d, 1 H) (3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 451 (MH^+, 99), 604 (8), 452 (26), 451 (99), 407 (10), 236 (17), 192 (9), 102 (9), 88 (45), 86 (9), 43 (9). Anal. ($C_{22}H_{30}N_2O_8$·0.72H_2O) C, H, N.

***tert*-Butyl (1*S*)-1-(4-Hydroxy-3-iodobenzyl)-2-oxo-2-(pentylamino)ethylcarbamate (53).** To a stirring mixture of 3-iodo-L-tyrosine **52** (10.0 g, 32.6 mmol) in dioxane (100 mL), H_2O (50 mL) and 1 M aqueous NaOH (50 mL) was added di-*tert*-butyl dicarbonate (7.8 g, 35.8 mmol) at 0 °C. The mixture was stirred for 2 h, and the solution, being allowed to warm to ambient temperature, was then washed with EtOAc (2 × 50 mL). The water layer was separated and carefully acidified with 4 M $NaHSO_4$·H_2O in a beaker and was then extracted with EtOAc (2 × 100 mL). The organic layer was dried ($NaSO_4$) and concentrated to afford 15.1 g (>100%) of the crude acid as a yellow oil. The acid was suspended in CH_2Cl_2 (200 mL) and cooled with ice to 0 °C. EDC (6.2 g, 32.6 mmol) was added and the mixture was stirred for 10 min at 0 °C. 1-Pentylamine (3.8 mL, 32.6 mmol) was added, and the mixture was stirred for 16 h, the solution being allowed to warm to ambient temperature. The mixture was washed with 10% aqueous HCl (2 × 100 mL) and the organic layer dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc/*n*-hexane 1:1), which furnished 10.0 g (64%) of **53** as a white solid: ^{1}H NMR 400 MHz ($CDCl_3$) δ 0.87 (t, 3H, J = 7.1, 14.4), 1.21 (m, 2H),1.30 (m, 2H), 1.41 (m, 2H), 1.43 (s, 9H), 2.93 (m, 2H), 3.09−3.20 (m, 2H), 4.17 (m, 1H), 5.20 (br m, 1H), 6.00 (m, 1H), 6.85 (d, 1H, J = 8.2), 7.05 (dd, 1H, J = 8.2, 2.0), 7.50 (d, 1H, J = 2.0).

Methyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-hydroxybenzoate (54). Triethylamine (0.61 mL, 4.41 mmol) was added to a stirring suspension of **53** (1.05 g, 2.20 mmol), palladium(II) acetate (14 mg, 0.066 mmol), and 1,1′-bis(diphenylphosphino)ferrocene (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 cooling 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 **54** 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).

Methyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)benzoate (55). A mixture of **54** (178 mg, 0.44 mmol), methyl bromoacetate (124 μL, 1.31 mmol), and freshly ground K_2CO_3 (181 mg, 1.31 mmol) was suspended in acetone (4 mL). The mixture was heated to 50 °C for 16 h. H_2O (5 mL) was added

and the mixture was extracted with EtOAc (3 mL). The organic layer was dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc/*n*-hexane 1:1), which furnished 192 mg (91%) of **55** as a white solid: ^{1}H NMR 500 MHz ($CDCl_3$) δ 0.87 (t, 3H, J = 7.1, 14.6), 1.21 (m, 2H), 1.28 (m, 2H), 1.38 (m, 2H), 1.41 (s, 9H), 3.00 (m, 2H), 3.16 (dd, 2H, J = 13.0, 6.6), 3.79 (s, 3H), 3.88 (s, 3H), 4.23 (dd, 1H, J = 14.1, 6.8), 4.70 (s, 2H), 5.10 (br t, 1H), 6.81 (d, 1H, J = 8.5), 7.29 (dd, 1H, J = 8.5, 2.2), 7.66 (d, 1H, J = 2.2).

4-{[(1*S*)-1-[3-(Methoxycarbonyl)-4-(2-methoxy-2-oxoethoxy)benzyl]-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoic Acid (56). Trifluoroacetic acid (0.38 mL, 4.98 mmol) was carefully added to a stirring solution of **55** (159 mg, 0.33 mmol) in CH_2Cl_2 (3 mL) at 0 °C. The mixture was stirred for 4 h, the solution being allowed 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 130 mg (quantitative) 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 (33 mg, 0.33 mmol) and triethylamine (101 μL, 0.73 mmol) were added, and the mixture was stirred for 16 h, the solution being allowed to warm to ambient temperature. The mixture was diluted with CH_2Cl_2 (3 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, 5% MeOH in CH_2Cl_2, then 5% MeOH/1% HOAc in CH_2Cl_2). 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 109 mg (69%) of **56** as a white solid: ^{1}H NMR 400 MHz (MeOH-d_4) δ 0.95 (t, 3H, J = 7.1, 14.4), 1.29 (m, 2H), 1.38 (m, 2H), 1.48 (m, 2H), 2.43−2.64 (m, 4H), 2.91 (dd, 1H, J = 13.8, 8.6), 3.13−3.22 (m, 3H), 3.82 (s, 3H), 3.92 (s, 3H), 4.55 (m, 1H), 4.83 (s, 2H), 7.00 (d, 1H, J = 8.6), 7.43 (dd, 1H, J = 8.6, 2.3), 7.72 (d, 1H, J = 2.3), 7.97 (br t, 1H).

2-(Carboxymethoxy)-5-[(2*S*)-2-[(3-carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]benzoic Acid (57). A solution of **56** (87 mg, 0.18 mmol) and 2.5 M aqueous LiOH (435 μL, 1.1 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 68 mg (83%) of **57** as a white solid: ^{1}H NMR 400 MHz (MeOH-d_4) δ 0.66 (t, 3H, J = 7.1, 14.4), 1.00 (m, 2H), 1.08 (m, 2H), 2.13−2.40 (m, 4H), 2.65 (dd, 1H, J = 13.8, 8.6), 2.84−2.93 (m, 3H), 4.28 (m, 1H), 4.58 (s, 2H), 6.79 (d, 1H, J = 8.6), 7.20 (dd, 1H, J = 8.6, 2.2), 7.56 (d, 1H, J = 2.2), 7.69 (br t, 1H); ^{13}C NMR (MeOH-d_4) δ 14.75, 23.78, 30.34, 30.47, 30.52, 31.74, 38.22, 40.87, 56.58, 67.67, 115.90, 121.69, 132.74, 134.38, 136.40, 158.14, 169.50, 172.65, 173.41, 174.97, 176.76; MS (ESI) 453 (M + H). Anal. ($C_{21}H_{28}O_9N_2 \cdot {}^1/_2H_2O$) C, H.

4-{[(1*S*)-1-[4-Hydroxy-3-(methoxycarbonyl)benzyl]-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoic Acid (58). Trifluoroacetic acid (0.48 mL, 6.2 mmol) was carefully added to a stirring solution of **54** (169 mg, 0.41 mmol) in CH_2Cl_2 (2 mL) at 0 °C. The mixture was stirred for 4 h, the solution being allowed 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 124 mg (98%) 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 (41 mg, 0.40 mmol) and triethylamine (124 μL, 0.89 mmol) were added, and the mixture was stirred for 16 h, the solution being allowed to warm to ambient temperature. The mixture was diluted with CH_2Cl_2 (8 mL) and the organic layer was washed with 10% aqueous HCl (2 × 3 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography as described for **56**, giving 111 mg (67%) of **58** as a white solid: ^{1}H NMR 500 MHz (DMSO) δ 0.83 (t, 3H, J = 7.2, 15.0), 1.17 (m, 2H), 1.24 (m, 2H) 1.30 (m, 2H), 2.25−2.38 (m, 2H), 2.69 (dd, 1H), 2.88 (dd, 1H), 2.93 (m, 1H), 3.06 (m, 1H), 3.90 (s, 3H), 4.37 (m, 1H), 6.88 (d, 1H, J = 8.5), 7.36 (dd, 1H, J = 2.1, 8.5), 7.64 (d, 1H, J = 2.1), 7.83 (t, 1H, J = 5.5, 11.2), 8.06 (d, 1H, J = 8.4), 10.35 (s, 1H).

5-[(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]-2-hydroxybenzoic Acid (59). A solution of **58** (101 mg, 0.25 mmol) and 2.5 M aqueous LiOH (395 μL, 0.99 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 93 mg (96%) of **59** as a white solid: ^{1}H NMR 400 MHz (MeOH-d_4) δ 0.89 (t, 3H, J = 7.1, 14.5), 1.19 (m, 2H), 1.29 (m, 2H), 1.41 (m, 2H), 2.43−2.60 (m, 4H), 2.86 (dd, 1H), 3.07 (m, 2H), 3.17 (m, 1H), 4.50 (m, 1H), 6.86 (d, 1H, J = 8.5), 7.38 (dd, 1H, J = 2.0, 8.5), 7.75 (d, 1H, J = 2.0), 7.92 (br m, 1H), 8,25 (br d, 1H); ^{13}C NMR (MeOH-d_4) δ 14.3, 23.3, 29.9, 30.0, 30.05, 31.3, 37.9, 40.4, 56.3, 114.1, 118.6, 129.5, 132.5, 138.0, 162.5, 173.5, 173.8, 174.9, 176.8. MS (ESI) m/z 395 (M + H). Anal. ($C_{21}H_{28}O_9N_2 \cdot {}^1/_3H_2O$) C, H.

Benzyl 5-[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]-2-hydroxybenzoate (60) Triethylamine (1.71 mL, 12.5 mmol) and benzyl alcohol (6.45 mL, 62 mmol) were added to a suspension of **53** (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 DMF (15 mL). The mixture was saturated with carbon monoxide (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. Crystallization in EtOAc/*n*-hexane gave 0.82 g (27%) of pure **60** as a white solid: ^{1}H 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 (1H, s).

Benzyl 2-(2-Amino-2-oxoethoxy)-5-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]benzoate (61). A mixture of **60** (295 mg, 0.61 mmol), 2-bromoacetamide (168 mg, 1.22 mmol), and freshly ground K_2CO_3 (168 mg, 1.22 mmol) was suspended in acetone (3 mL). The mixture was stirred at 40 °C for 16 h, after which time H_2O (5 mL) was added and the mixture was extracted with EtOAc (2 × 5 mL). The organic layer was washed with brine (4 mL), dried ($MgSO_4$), and concentrated in vacuo, which afforded 273 mg (83%) of **61** 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).

Benzyl 2-(2-Amino-2-oxoethoxy)-5-[(2*S*)-2-{[4-(benzyloxy)-4-oxobutanoyl]amino}-3-oxo-3-(pentylamino)propyl]benzoate (62). Trifluoroacetic acid (0.55 mL, 7.12 mmol) was carefully added to a stirring solution of **61** (257 mg, 0.47 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The mixture was stirred for 4 h, the solution being allowed 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 276 mg (>100%) of the crude amine as a colorless oil. The amine was dissolved in DMF (3 mL) and cooled with ice. Benzyl hydrogen succinate (109 mg, 0.52 mmol) and EDC (100 mg, 0.52 mmol) were added, and the mixture was stirred for 16 h, the solution being allowed to warm to ambient temperature. The mixture was concentrated and partitioned between EtOAc (3 mL) and 10% aqueous HCl (2 · 3 mL). The water layer was saturated with

NaCl and extracted three times with EtOAc. The collected organic layers were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (SiO_2, EtOAc), which furnished 93 mg (31%) of **62** as a white solid: ^{1}H NMR 400 MHz (MeOH-d_4) δ 0.88 (t, 3H, J = 7.1, 14.5), 1.18 (m, 2H), 1.27 (m, 2H) 1.39 (m, 2H), 2.46 (m, 2H), 2.59 (m, 2H), 2.83 (dd, 1H), 3.09 (m, 3H), 4.51 (dd, 1H, J = 6.3, 8.4), 4.55 (s, 2H), 5.10 (s, 2H), 5.35 (s, 2H), 7.04 (d, 1H, J = 8.5), 7.3−7.5 (m, 11H), 7.80 (d, 1H, J = 2.4), 7.89 (br t, 0.5H), 8.00 (s, 1H).

(2*S*)-(2-Amino-2-oxoethoxy)-5-[(2*S*)-2-[(3-carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]benzoic Acid (63). A mixture of **62** (80 mg, 0.13 mmol) and 5% Pd/C (25 mg) in methanol (4 mL) was hydrogenated at atmospheric pressure for 3 h. The mixture was filtered through Celite and solvent removed in vacuo to afford 42 mg (71%) of **63** as a white solid: ^{1}H NMR 400 MHz (MeOH-d_4) δ 0.90 (t, 3H, J = 7.1, 14.4), 1.22 (m, 2H), 1.31 (m, 2H) 1.43 (m, 2H), 2.4−2.6 (m, 4H), 2.58 (m, 2H), 2.88 (dd, 1H, J = 8.5, 14.8), 3.11 (m, 1H), 4.53 (dd, 1H, J = 6.3, 8.5), 4.59 (s, 2H), 7.02 (d, 1H, J = 8.5), 7.40 (dd, 1H, J = 2.2, 8.5) 7.75 (d, 1H, J = 2.2), 7.96 (br t, 0.5H); ^{13}C NMR (MeOH-d_4) δ 14.7, 23.8, 30.3, 30.5, 30.7, 31.9, 38.3, 40.8, 56.7, 69.2, 115.5, 123.7, 132.3, 133.9, 135.5, 157.8, 170.7, 173.5, 174.6, 175.0, 176.9. MS (ESI) m/z 452 (M + H). Anal. ($C_{21}H_{29}O_8N_3$·H_2O) C, H, N.

***tert*-Butyl (1*S*)-1-[3-(Aminocarbonyl)-4-hydroxybenzyl]-2-oxo-2-(pentylamino)-ethylcarbamate (64).** In a 50 mL dried round-bottom flask were combined **53** (1.12 g, 2.34 mmol), palladium(II) acetate (16 mg, 3 mol %), and 1,1′-bis-(diphenylphosphino)ferrocene (dppf, 78 mg, 6 mol %). DMF (5 mL) was added and a carbon monoxide atmosphere was established in the reaction vessel. Diisopropylethylamine (0.82 mL, 4.69 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (3.46 mL, 16.4 mmol) were added, and the reaction vessel was again purged with successive cycles of carbon monoxide and vacuum (five times). The mixture was stirred at 90 °C for 16 h. After being cooled to ambient temperature, the reaction mixture was acidified with 1 M aqueous HCl (pH 3−4) and stirred for about 5−10 min. The solution was then basified with 10 M aqueous NaOH and extracted with EtOAc (2 × 5 mL). The organic layer was dried ($MgSO_4$) and concentrated. The resulting residue was purified by flash chromatography (SiO_2, EtOAc/*n*-hexane 2:1), which furnished 552 mg (60%) of **64** as a white solid: ^{1}H NMR 400 MHz (MeOH-d_4) δ 0.89 (t, 3H, J = 7.2, 14.5), 1.17 (m, 2H), 1.28 (m, 2H), 1.38 (m, 2H), 1.40 (s, 9H), 2.80 (dd, 1H, J = 7.9, 13.9), 2.98 (dd, 1H, J = 7.6, 13.9), 3.05 (m, 1H), 3.18 (m, 1H), 4.22 (br t, 1H), 6.84 (d, 1H, J = 8.5), 7.28 (dd, 1H, J = 2.2, 8.5), 7.62 (d, 1H, J = 2.2).

Methyl {2-(Aminocarbonyl)-4-[(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}-acetate (65). A mixture of **64** (340 mg, 0.86 mmol), methyl bromoacetate (123 μL, 1.30 mmol), and freshly grounded K_2CO_3 (179 mg, 1.30 mmol) was suspended in acetone (7 mL). The mixture was stirred at room temperature for 16 h, after which time H_2O (5 mL) was added and the mixture was extracted with EtOAc (2 × 5 mL). The organic layer was washed with brine (4 mL), dried ($MgSO_4$), and concentrated in vacuo, which afforded 370 mg (92%) of **65** as a white solid, pure enough (>95%) to use in the next step without further purification: ^{1}H NMR 400 MHz ($CDCl_3$) δ 0.86 (t, 3H, J = 7.1, 14.3), 1.19 (m, 2H), 1.27 (m, 2H), 1.39 (s, 9H), 1.36−1.40 (m, 2H), 2.98 (dd, 1H, J = 7.0, 13.8), 3.09 (dd, 1H, J = 6.8, 13.8), 3.17 (m, 2H), 4.27 (br m, 1H), 5.08 (br m 1H), 5.87 (br m, 1H), 5.98 (br m, 1H), 6.78 (d, 1H, J = 8.5), 7.34 (br dd, 1H), 8.08 (d, 1H, J = 1.8), 8.32 (br s, 1H).

4-{[(1*S*)-1-[3-(Aminocarbonyl)-4-(2-methoxy-2-oxoethoxy)benzyl]-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoic Acid (66). Trifluoroacetic acid (0.74 mL, 9.63 mmol) was carefully added to a stirring solution of **65** (299 mg, 0.64 mmol) in CH_2Cl_2 (5 mL) at 0 °C. The mixture was stirred for 3 h, the solution being allowed to warm to ambient temperature. The volatiles were removed in vacuo, and the residue was partitioned between EtOAc (4 mL) and saturated aqueous $NaHCO_3$ (3 mL). The organic layer was dried ($MgSO_4$), and concentrated to dryness to afford 196 mg (84%) of the crude amine as a brown/yellow oil. The amine was dissolved in CH_2Cl_2 (6 mL) and cooled with ice. Succinic anhydride (54 mg, 0.54 mmol) and triethylamine (164 μL, 1.18 mmol) were added, and the mixture was stirred for 16 h, the solution being allowed to warm to ambient temperature. The mixture was diluted with CH_2Cl_2 (10 mL), and the organic layer was washed with 10% aqueous HCl (2 × 5 mL), dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography as described for **56**, which furnished 83 mg (33%) of **66** as a white solid: ^{1}H NMR 400 MHz (DMSO) δ 0.95 (t, 3H, J = 7.0, 14.3), 1.28 (m, 2H), 1.36 (m, 2H), 1.43 (m, 2H), 2.35−2.49 (m, 4H), 2.82 (dd, 1H), 3.0−3.18 (m, 3H), 3.85 (s, 3H), 4.48 (m, 1H), 5.04 (s, 2H), 7.09 (d, 1H, J = 8.5), 7.42 (br d, 1H, J = 8.5), 7.74 (br s, 0.3H), 7.93 (br s, 1H), 8.01 (br m, 0.5H), 8.08 (br s, 0.4H), 8,24 (br d, 0.5H). Anal.($C_{22}H_{31}O_8N_2$·$0.5H_2O$) C, H.

4-{[(1*S*)-1-[3-(Aminocarbonyl)-4-(carboxymethoxy)-benzyl]-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoic Acid (67). A solution of **66** (42 mg, 0.09 mmol) and 2.5 M aqueous LiOH (72 μL, 0.18 mmol) in THF/MeOH/H_2O 3:1:1 (1 mL) was stirred at ambient temperature for 16 h. The reaction mixture was extracted with EtOAc (2 mL), and the water layer 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 21 mg (42%) of **67** as a white solid: ^{1}H NMR 400 MHz (MeOH-d_4) δ 0.90 (t, 3H, J = 7.1, 14.5), 1.22 (m, 2H), 1.31 (m, 2H), 1.41 (m, 2H), 2.40−2.62 (m, 4H), 2.89 (dd, 1H, J = 8.8, 13.8), 3.08−3.19 (m, 3H), 4.53 (dd, 1H, J = 6.1, 8.8), 4.82 (s, 2H), 7.03 (d, 1H, J = 8.5), 7.41 (dd, 1H, J = 2.4, 8.5), 7.93 (d, 1H, J = 2.4), 8.28 (d, 0.3H, J = 7.9); ^{13}C NMR (MeOH-d_4) δ 14.7, 23.8, 30.2, 30.3, 30.5, 31.8, 38.3, 40.8, 56.6, 67.3, 114.7, 122.8, 132.4, 139.0, 135.6, 157.2, 169.9, 172.4, 173.5, 175.0, 176.8; MS (ESI) m/z 452 (M + H).

Methyl 5-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)benzoate (68). Compound **55** (0.50 g, 1.0 mmol) was dissolved in a 4 M solution of HCl in dioxane (5 mL). After stirring at room temperature for 2 h, the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (5 mL) and triethylamine (0.43 mL, 3.1 mmol), and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (0.28 g, 1.0 mmol) was added. After the mixture was cooled to 0 °C, EDC (0.20 g, 1.0 mmol) and HOBT (0.14 g, 1.0 mmol) were added. The mixture was warmed to room temperature and stirred overnight. The mixture was partitioned between EtOAc and 1 M HCl. The organic phase was washed with sat. $NaHCO_3$ and sat. NaCl, dried ($MgSO_4$), and concentrated to a glassy solid (0.54 g). The residue was purified by flash chromatography (40 g SiO_2, 60% EtOAc/heptane) to obtain 0.42 g (67%) of **68** as a white powder: ^{1}H NMR ($CDCl_3$) δ 7.48 (br s, 1 H), 7.30 (m, 3 H), 7.18 (m, 3 H), 6.76 (d, 1 H), 6.32 (br s, 1 H), 6.00 (br s, 1 H), 4.90 (br s, 1 H), 4.68 (s, 3 H), 4.55 (m, 1 H), 4.25 (m, 1 H), 3.88 (s, 3 H), 3.78 (s, 3 H), 3.07 (m, 4 H), 2.90 (m, 2 H), 1.40−1.20 (m, 15 H), 0.86 (t, 3 H).

5-[(2*S*)-2-({(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-3-phenylpropanoyl}amino) 3-oxo-3-(pentylamino)propyl]-2-(carboxymethoxy)benzoic acid (69) was prepared by saponification of **68** with LiOH as described in the preparation of **38**: ^{1}H NMR (DMSO-d_6) δ 7.92 (br s, 1 H), 7.54 (br s, 1 H), 7.30 (d, 1 H), 7.18 (m, 6 H), 6.90 (d, 1 H), 4.68 (s, 2 H), 4.40 (m, 1 H), 4.05 (m, 1 H), 2.98 (m, 2 H), 2.83 (m, 2 H), 2.65 (m, 2 H), 1.33−1.10 (m, 15 H), 0.82 (t, 3 H); MS (FAB) m/z (rel intensity) 600 (MH^+, 21), 622 (15), 600 (21), 501 (30), 500 (99), 238 (11), 120 (73), 88 (34), 57 (52), 43 (12), 41 (14); HRMS (FAB) calcd for $C_{31}H_{41}N_3O_9$ + H 600.2921, found 600.2923. Anal. ($C_{31}H_{41}N_3O_9$·$0.38H_2O$) C, H, N.

Methyl 5-[(2*S*)-2-({(2*S*)-2-[(4-methoxy-4-oxobutanoyl)-amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(2-methoxy-2-oxoethoxy)benzoate (70) was prepared by removal of the Boc group from **68** with HCl/dioxane followed by coupling with monomethyl succinate with EDC and HOBT as described for the preparation of **13** from **11a**: ^{1}H NMR ($CDCl_3$) δ 7.69 (d, J = 2 Hz, 1 H), 7.35 (dd, J = 8, 2 Hz, 1 H), 7.23 (m, 4 H), 7.10 (m, 2 H), 6.80 (d, J = 8 Hz,

1 H), 6.51 (d, $J = 7$ Hz, 1 H), 6.40 (bt, $J = 6$ Hz, 1 H), 4.71 (s, 2 H), 4.56 (m, 2 H), 3.92 (s, 3 H), 3.77 (s, 3 H), 3.64 (s, 3 H), 3.35 (dd, $J = 4$, 15 Hz, 1 H), 3.15 (m, 3 H), 2.95 (m, 2 H), 2.2−2.7 (m, 4 H), 1.45 (m, 2 H), 1.2−1.4 (m, 4 H), 0.89 (t, $J = 7$ Hz, 3 H); MS (FAB) *m/z* (rel intensity) 642 (MH^+, 89), 643 (32), 642 (89), 234 (39), 120 (99), 115 (61), 73 (32), 57 (43), 55 (29), 43 (44), 41 (26); HRMS (FAB) calcd for $C_{33}H_{43}N_3O_{10}$ + H 642.3026, found 642.3032. Anal. ($C_{33}H_{43}N_3O_{10}$·0.37H_2O) C, H, N.

2-(Carboxymethoxy)-5-[(2*S*)-2-({(2*S*)-2-[(3-carboxypropanoyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]benzoic acid (71) was prepared by saponification of **70** with LiOH as described for the preparation of **38**: ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1 H), 8.00 (d, 1 H), 7.78 (t, 1 H), 7.53 (d, 1 H), 7.29 (dd, 1 H), 7.16 (m, 5 H), 6.90 (d, 1 H), 4.71 (s, 2 H), 4.37 (m, 2 H), 2.95 (m, 4 H), 2.70 (m, 2 H), 2.29 (m, 4 H), 1.35−1.15 (m, 6 H), 0.82 (t, 3 H); MS (FAB) *m/z* (rel intensity) 600 (MH^+, 20), 600 (20), 353 (12), 248 (11), 238 (12), 220 (15), 131 (9), 121 (10), 120 (99), 88 (32), 43 (11); HRMS (FAB) calcd for $C_{30}H_{37}N_3O_{10}$ + H 600.2557, found 600.2564. Anal. ($C_{30}H_{37}N_3O_{10}$·0.87H_2O) C, H, N.

2-{4-[(2*R*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (72) was prepared by the method described for the synthesis of **8** from *N*-Boc-D-tyrosine: MS (ES−) 451. Anal. $C_{21}H_{28}N_2O_9$·0.64H_2O) C, H, N.

4-{[(1*S*)-1-{4-[2-Ethoxy-1-(ethoxycarbonyl)-2-oxoethoxy]benzyl}-2-oxo-2-(pentylamino)ethyl]amino}-4-oxobutanoic Acid (73). To a solution of **13b** ($R^3 = CH_2CH_2CO_2Bn$) (79 mg, 0.145 mmol) in MeOH (2 mL) was added 10% Pd/C (8 mg). The mixture was stirred at room temperature under an atmosphere of hydrogen (balloon) for 1 h. The mixture was filtered through Celite and concentrated. The resulting oil was dissolved in CH_2Cl_2 (0.5 mL) and cooled to 0 C. Succinic anhydride (14 mg, 0.145 mmol) was added, and the mixture was stirred at room temperature overnight. The mixture was diluted with EtOAc (20 mL) and washed with 0.5 M HCl (10 mL) and sat. NaCl (10 mL). After drying ($MgSO_4$), the organic phase was concentrated to provide 71 mg (96%) of **73** as a glass: ^{1}H NMR ($CDCl_3$) δ 7.12 (d, $J = 9$ Hz, 2 H), 6.87 (d, $J = 9$ Hz, 2 H), 6.60 (br s, 1 H), 5.80 (br s, 1 H), 5.17 (s, 1 H), 4.52 (dd, $J =$ Hz, 1 H), 4.32 (m, 4 H), 3.14 (dd, $J = 13$, 6.5 Hz, 2 H), 2.96−3.03 (m, 2 H), 2.63−2.67 (m, 2 H), 2.45−2.50 (m, 2 H), 1.17−1.42 (m, 12 H), 0.87 (t, $J = 7$ Hz, 3 H); MS (FAB) *m/z* 509 (MH^+), 531, 510, 509, 391, 294, 265, 88, 86, 43, 29;

HRMS (FAB) calcd for $C_{25}H_{36}N_2O_9$ + H 509.2499, found 509.2494.

4-Oxo-4-{[(1*S*)-2-oxo-2-(pentylamino)-1-(4-{[(trifluoromethyl)sulfonyl]oxy}benzyl)ethyl]amino}butanoic Acid (74). The title compound was prepared as described for **42** using trifluoromethanesulfonic anhydride, but without the use of methanol in the hydrolysis. After the workup, a white solid was obtained. Recrystallization from acetonitrile gave an amorphous solid (43%): mp 140.2−142.7 °C; ^{1}H NMR (CD_3-OD) δ 0.91 (t, $J = 7.3$, 3H), 1.19−1.37 (m, 4H), 1.40−1.47 (m, 2H), 2.37−2.70 (m, 4H), 2.95 (dd, $J_{1,2} = 8.7$, 1H), 3.07−3.24 (m, 3H), 4.58 (dd, $J_1 = 8.9$, $J_2 = 8.7$, 1H), 7.29 (d, $J = 8.7$, 2H), 7.41 (d, $J = 8.7$, 2H); ^{13}C NMR (CD_3OD) δ 15.52, 24.54, 31.11, 31.23, 31.28, 32.50, 39.31, 41.56, 57.11, 121.33 (q, $J = 319.8$, CF_3), 123.51, 133.56, 141.48, 151.07, 174.01, 175.74, 177.54; MS (ionspray, $[M - H]^-$) *m/z* 481.4. Anal. ($C_{19}H_{25}F_3N_2O_7S$) H, N; C: calcd, 47.30; found, 48.03.

2-{4-[(2*S*)-2-{[4-(Benzyloxy)-4-oxobutanoyl]amino}-3-oxo-3-(pentylamino)propyl]phenoxy}malonic Acid (75). Trifluoroacetic acid (5 mL) was added to a solution of **7** (225 mg, 0.344 mmol) in methylene chloride (5 mL) with ice-bath chilling. The solution was stirred at room temperaturefor 2 h. Concentration in vacuo afforded the title compound as a light amber foam: ^{1}H NMR (CD_3OD) 7.33 (m, 5 H), 7.15 (d, $J = 9$ Hz, 2 H), 6.89 (d, $J = 9$ Hz, 2 H), 5.23 (s, 1 H), 5.10 (s, 2 H), 4.48 (m, 1 H), 3.05 (m and dd obscured, 3 H), 2.80 (dd, $J = 7$, 14 Hz, 1 H), 2.35−2.7 (m, 4 H), 1.1−1.5 (m, 6 H), 0.88 (t, $J = 7$ Hz, 3 H); MS (FAB) *m/z* (rel intensity) 543 (M + H, 45), 544 (14), 543 (45), 542 (5), 441 (6), 92 (9), 91 (99), 88 (23), 86 (6), 43 (6); HRMS (FAB) calcd for $C_{28}H_{34}N_2O_9$ + H 543.2343, found 543.2332.

2-{4-[(2*S*)-2-[(4-Amino-4-oxobutanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic Acid (76). To a suspension of succinamic acid (40 mg, 0.34 mmol) in CH_2Cl_2 (1 mL) at 0 °C was added EDC (65 mg, 0.34 mmol) and HOBT (46 mg, 0.34 mmol), and the mixture was stirred for a few minutes. Compound **12b** (150 mg, 0.34 mmol) and triethylamine (48 mL, 0.34 mmol) were added, and the mixture was warmed to room temperature and stirred overnight. The mixture was partitioned between EtOAc and 1 M HCl. The organic phase was washed with sat. $NaHCO_3$ and sat. NaCl and dried ($MgSO_4$). After the solvent was removed under reduced pressure, the residue was purified by flash chromatography (11 g SiO_2, 6% MeOH/CH_2Cl_2) to yield 72 mg of a white solid. To a solution of the solid dissolved in THF (3 mL) and MeOH (1 mL) was added a solution of LiOH·H_2O (30 mg, 0.71 mmol) in H_2O (1 mL). The mixture was stirred at room temperature for 2.5 h. The mixture was neutralized with 1 M HCl and extracted with EtOAc (3×). The combined organic phase was washed with sat. NaCl and dried ($MgSO_4$). The solvent was removed in vacuo to give 27 mg (18%) of **76** as a white solid: ^{1}H NMR (DMSO) δ 8.05 (d, 1 H), 7.85 (t, 1 H), 7.27 (br s, 1 H), 1.12 (d, 2 H), 6.79 (d, 2 H), 6.75 (br s, 1 H), 5.26 (s, 1 H), 4.30 (m, 1 H), 2.89−3.03 (m, 3 H), 2.89−3.03 (m, 3 H), 2.60−2.71 (m, 1 H), 2.12−2.32 (m, 4 H), 1.11−1.38 (m, 6 H), 0.83 (t, 3 H); MS (ES−) 450; HRMS (FAB) calcd for $C_{21}H_{29}N_3O_8$ + H 452.2032; found 452.2051.

2-{4-[(2*S*)-2-[(4-Carboxybutanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (77) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.84 (t, 3 H), 1.15−1.36 (m, 6 H), 1.61 (t, 2 H), 2.06 (m, 4 H), 2.68 (m, 1 H), 2.84 (m, 1 H), 3.00 (m, 2 H), 4.38 (m, 1 H), 5.25 (s, 1 H), 6.80 (d, 2 H), 7.13 (d, 2 H), 7.86 (t, 1 H), 8.00 (d, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 467 (MH^+, 99), 481 (18), 468 (24), 467 (99), 423 (20), 238 (15), 194 (14), 91 (13), 88 (72), 59 (14), 43 (16). Anal. ($C_{22}H_{30}N_2O_9$·0.82H_2O) C, H, N.

2-{4-[(2*S*)-2-{[2-(2-Hydroxy-2-oxoethoxy)acetyl]amino}-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (78) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.83 (t, 3 H), 1.20−1.32 (m, $J =$ Hz, 6 H), 2.76−3.01 (m, 4 H), 3.89 (s, 2 H), 4.03 (s, 2 H), 4.45 (m, 1 H), 5.23 (s, 1 H), 6.78 (d, 2 H), 7.08 (d, 2 H), 7.77 (d, 1 H), 7.95 (t, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 469 (MH^+, 22), 497 (40), 483 (53), 469 (22), 107 (22), 88 (99), 86 (22), 75 (22), 43 (52), 39 (22), 23 (28). Anal. ($C_{21}H_{28}N_2O_{10}$·0.89H_2O) C, H, N.

2-{4-[(2*S*)-2-[(2-Carboxyacetyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (79) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.19−1.31 (m, 6 H), 2.69 (m, 1 H), 2.85−3.21 (m, 5 H), 4.37 (m, 1 H), 5.23 (s, 1 H), 6.78 (d, 2 H), 7.09 (d, 2 H), 7.86 (t, 1 H), 8.27 (d, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 439 (MH^+, 99), 453 (41), 440 (13), 439 (99), 395 (10), 331 (18), 177 (81), 133 (13), 118 (35), 88 (94), 23 (17). Anal. ($C_{20}H_{26}N_2O_9$·0.73H_2O) C, H, N.

2-{4-[(2*S*)-2-[(4-Carboxy-3,3-dimethylbutanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (80) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.85 (m, 9 H), 1.11−1.40 (m, 6 H), 2.12 (m, 4 H), 2.67 (m, 1 H), 2.85 (m, 1 H), 2.98 (m, 2 H), 5.22 (m, 1 H), 5.73 (s, 1 H), 6.78 (d, 2 H), 7.11 (d, 2 H), 7.84 (t, 1 H), 7.97 (d, 1 H) 3-CO_2H absent). Anal. ($C_{24}H_{34}N_2O_9$·1.27H_2O) C, H, N.

2-{4-[(2*S*)-2-({2-[1-(Carboxymethyl)cyclopentyl]acetyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (81) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.20−1.47 (m, 14 H), 2.18−2.35 (m, 4 H), 2.60−2.92 (m, 2 H), 2.99 (q, 2 H), 4.40 (m, 1 H), 5.22 (s, 1 H), 7.79 (d, 2 H), 7.11 (d, 2 H), 7.83 (t, 1 H), 8.00 (d, 1 H) (3-CO_2H absent);

MS (FAB) *m/z* (rel intensity) 462 (MH^+, 0), 535 (48), 521 (36), 143 (36), 136 (33), 109 (40), 88 (99), 81 (83), 67 (37), 43 (42), 39 (37). Anal. ($C_{26}H_{36}N_2O_9 \cdot H_2O$) C, H, N.

2-{4-[(2*S*)-2-({[(1*R*,2*S*)-2-Carboxycyclohexyl]carbonyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (82) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.84 (m, 3 H), 1.12−1.50 (m, 14 H), 1.80 (m, 1 H), 2.4−3.15 (m, 4 H), 3.35 (m, 1 H), 5.22 (m, 1 H), 6.65 (d, 0.2 H), 6.78 (d, 1.8 H), 7.0 (d, 0.2 H), 7.08 (m, 1.8 H), 7.22 (m, 1 H), 7.62 (m, 1 H), 7.75 (d, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 507 (MH^+, 99), 508 (28), 507 (99), 238 (42), 194 (38), 136 (26), 102 (37), 91 (31), 88 (78), 81 (66), 43 (30). Anal. ($C_{25}H_{34}N_2O_9 \cdot 0.87H_2O$) C, H, N.

2-{4-[(2*S*)-2-({[(1*R*,2*R*)-2-Carboxycyclohexyl]carbonyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (83) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.82 (m, 3 H), 1.20 (m, 8 H), 1.53−1.96 (m, 4 H), 2.40 (m, 2 H), 2.54−2.89 (m, 2 H), 3.00 (m, 4 H), 4.29 (m, 1 H), 5.23 (m, 1 H), 6.76 (d, 2 H), 7.08 (m, 2 H), 7.52 (m, 1 H), 7.90 (m, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 507 (MH^+, 99), 508 (29), 507 (99), 353 (35), 238 (24), 194 (19), 143 (18), 109 (15), 88 (63), 81 (41), 43 (17). Anal. ($C_{25}H_{34}N_2O_9 \cdot 1.6H_2O$) C, H, N.

2-{4-[(2*S*)-2-{[(2-Carboxycyclopropyl)carbonyl]amino}-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (84) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.22 (m, 8 H), 1.92 (m, 2 H), 2.72 (m, 1 H), 2.98 (m, 3 H), 4.32 (m, 1 H), 5.24 (s, 1 H), 6.78 (d, 2 H), 7.09 (m, 2 H), 7.82 (m, 1 H), 8.40 (m, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 465 (MH^+, 99), 466 (27), 465 (99), 421 (18), 238 (10), 194 (12), 113 (15), 107 (24), 102 (11), 88 (39), 63 (11). Anal. ($C_{22}H_{28}N_2O_9 \cdot 0.88H_2O$) C, H, N.

2-{4-[(2*S*)-2-[(2-Carboxybenzoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (85) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.83 (t, 3 H), 1.22 (m, 4 H), 1.40 (m, 2 H), 2.73 (dd, 1 H), 3.08 (m, 3 H), 4.50 (m, 1 H), 5.27 (s, 1 H), 6.83 (d, 2 H), 6.97 (d, 1 H), 7.15 (d, 2 H), 7.50 (m, 2 H), 7.70 (t, 1 H), 7.89 (d, 1 H), 8.52 (d, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 501 (MH^+, 54), 501 (54), 154 (64), 149 (23), 137 (71), 117 (33), 109 (30), 92 (22), 59 (99), 45 (24), 41 (24). Anal. ($C_{25}H_{28}N_2O_9 \cdot 1.28H_2O$) C, H, N.

2-{4-[(2*S*)-2-({[(1*R*,4*R*)-3-Carboxybicyclo[2.2.1]hept-2-yl]carbonyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (86) was prepared by the general procedure described for the preparation of **14** from **12a**: ^{1}H NMR (DMSO) δ 0.80 (m, 4 H), 1.21 (m, 7 H), 1.43−2.10 (m, 2 H), 2.12−2.42 (m, 2 H), 2.60−3.10 (m, 8 H), 4.34 (m, 1 H), 5.15 (m, 1 H), 6.75 (m, 2 H), 7.05 (m, 2 H), 7.60 (m, 1 H), 7.80 (m, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 519 (MH^+, 99), 519 (99), 353 (53), 238 (31), 194 (32), 177 (40), 167 (34), 88 (61), 67 (48), 43 (47), 23 (45). Anal. $C_{26}H_{34}N_2O_9 \cdot 0.90H_2O$) C, H, N.

2-(4-{(2*S*)-3-(Hexylamino)-2-[(4-hydroxy-4-oxobutanoyl)amino]-3-oxopropyl}phenoxy)malonic acid (87) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.19 (m, 8 H), 2.32 (m, 4 H), 2.66 (m, 1 H), 2.90 (m, 3 H), 4.32 (m, 1 H), 5.22 (s, 1 H), 6.76 (d, 2 H), 7.09 (d, 2 H), 7.77 (t, 1 H), 8.03 (d, 1 H) (3-CO_2H absent); HRMS (FAB) calcd for $C_{22}H_{30}N_2O_9$ + H 467.2029, found 467.2040. Anal. ($C_{22}H_{30}N_2O_9 \cdot 0.59H_2O$) C, H, N.

2-(4-{(2*S*)-3-(Butylamino)-2-[(3-carboxypropanoyl)amino]-3-oxopropyl}phenoxy)malonic acid (88) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (CD_3OD) δ 0.85 (t, 3H), 1.17−1.26 (m, 2H), 1.33−1.40 (m, 2H), 2.29−2.58 (m, 4H), 2.78 (dd, 1H), 3.01−3.11 (m, 3H), 4.44 (dd, 1H), 5.12 (s, 1H), 6.86 (d, 2H), 7.11 (d, 2H);^{13}C NMR (CD_3OD) δ 14.50, 21.38, 30.54, 31.78, 32.73, 38.39, 40.57, 40.70, 56.58, 116.67, 131.74, 132.34, 157.96, 165.04, 173.80, 175.01, 176.83; MS (ionspray, $[M + H]^+$) *m/z* 437.2. Anal. ($C_{20}H_{26}N_2O_9 \cdot 1.5H_2O$) C, H, N.

2-{4-[(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-(decylamino)-3-oxopropyl]phenoxy}malonic acid (89) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.06 (d, 1 H), 7.80 (t, 1 H), 7.10 (d, 2 H), 6.78 (d, 2 H), 5.24 (s, 1 H), 3.33 (q, 1 H), 2.98 (m, 2 H), 2.85 (dd, 1 H), 2.65 (dd, 1 H), 2.30 (m, 4 H), 1.21 (m, 16 H), 0.83 (t, 3 H); HRMS (FAB) calcd for $C_{26}H_{38}N_2O_9$ + H 523.2655; found 523.2634. Anal. ($C_{26}H_{38}N_2O_9$) C, H, N.

2-(4-{(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-[(cyclohexylmethyl)amino]-3-oxopropyl}phenoxy)malonic acid (90) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (DMSO) δ 0.78 (m, 2 H), 1.12 (m, 3 H), 1.32 (m, 1 H), 1.62 (m, 5 H), 2.30 (m, 4 H), 2.62−2.92 (m, 4 H), 4.32 (m, 1 H), 5.22 (s, 1 H), 6.78 (d, 2 H), 7.09 (m, 2 H), 7.76 (t, 1 H), 8.02 (d, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 479 (MH^+, 99), 480 (20), 479 (99), 435 (26), 331 (15), 177 (46), 127 (19), 114 (48), 71 (12), 57 (12), 55 (17); HRMS (FAB) calcd for $C_{23}H_{30}N_2O_9$ + H 479.2029, found 479.2029. Anal. ($C_{23}H_{30}N_2O_9 \cdot 0.49H_2O$) C, H, N.

2-{4-[(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-(isopentylamino)-3-oxopropyl]phenoxy}malonic acid (91) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (DMSO) δ 0.83 (d, 6 H), 1.22 (m, 2 H), 1.48 (m, 1 H), 2.32 (m, 4 H), 2.75 (m, 1 H), 2.82 (m, 1 H), 3.00 (m, 2 H), 4.35 (m, 1 H), 5.22 (s, 1 H), 6.78 (d, 2 H), 7.09 (d, 2 H), 7.74 (t, 1 H), 8.04 (d, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 453 (MH^+, 22), 475 (20), 453 (22), 431 (15), 194 (18), 136 (18), 88 (99), 86 (15), 55 (14), 43 (30), 23 (43). Anal. ($C_{21}H_{28}N_2O_9 \cdot 0.60H_2O$) C, H, N.

2-(4-{(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-[(3-phenylpropyl)amino]propyl}phenoxy)malonic acid (92) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.10 (d, 1 H), 7.89 (t, 1 H), 7.25 (t, 2 H), 7.15 (m, 5 H), 6.80 (d, 2 H), 5.23 (s, 1 H), 4.33 (m, 1 H), 3.01 (m, 2 H), 2.89 (dd, 1 H), 2.70 (m, 1 H), 2.32 (m, 4 H), 1.61 (m, 2 H); MS (ES−) 499. Anal. ($C_{25}H_{28}N_2O_9 \cdot 0.73H_2O$) C, H, N.

2-(4-{(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-[(1-naphthylmethyl)amino]-3-oxopropyl}phenoxy)malonic acid (93) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.45 (t, 1 H), 8.17 (d, 2 H), 8.00 (m, 1 H), 7.92 (m, 1 H), 7.81 (d, 1 H), 7.41 (m, 2 H), 7.41 (t, 1 H), 7.25 (d, 1 H), 7.11 (d, 2 H), 6.77 (d, 2 H), 5.25 (s, 1 H), 4.70 (d, 2 H), 4.50 (m, 1 H), 3.94 (dd, 1 H), 2.72 (dd, 1 H), 2.32 (m, 4 H); MS (ES−) 521; HRMS (FAB) calcd for $C_{27}H_{26}N_2O_9$ + H 523.1716, found 523.1722.

2-(4-{(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-[(3,3-diphenylpropyl)amino]-3-oxopropyl}phenoxy)malonic acid (94) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.04 (d, 1 H), 7.90 (t, 1 H), 7.25 (m, 8 H), 7.12 (m, 4 H), 6.77 (d, 2 H), 5.22 (s, 1 H), 4.34 (q, 1 H), 3.92 (t, 1 H), 2.89 (m, 3 H), 2.65 (m, 1 H), 2.31 (m, 4 H), 2.10 (q, 2 H); MS (ES+) 577. Anal. ($C_{31}H_{32}N_2O_9 \cdot 0.9H_2O$) C, H, N.

2-(4-{(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-[(2,2-diethoxyethyl)amino]-3-oxopropyl}phenoxy)malonic acid (95) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (DMSO) δ 1.07 (m, 6 H), 2.30 (m, 1 H), 2.66 (m, 1 H), 2.85 (m, 1 H), 3.15 (m, 2 H), 3.42 (m, 2 H), 3.55 (m, 2 H), 4.38 (m, 4 H), 5.25 (s, 1 H), 5.73 (s, 1 H), 6.78 (d, 2 H), 7.12 (d, 2 H), 7.92 (m, 1 H), 8.06 (m, 1 H) (3-CO_2H absent); MS (FAB) *m/z* (rel intensity) 499 (MH^+, 8), 453 (80), 251 (99), 194 (45), 177 (53), 136 (66), 107 (38), 101 (44), 88 (89), 57 (61), 23 (81). Anal. ($C_{22}H_{30}N_2O_{11} \cdot 1.20H_2O$) C, H, N.

2-(4-{(2*S*)-3-{[4-(Aminosulfonyl)phenethyl]amino}-2-[(3-carboxypropanoyl)amino]-3-oxopropyl}phenoxy)-malonic acid (96) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.09 (d, 1 H), 8.00 (t, 1 H), 7.71 (d, 2 H), 7.35 (d, 2 H), 7.27 (s, 2 H), 5.27 (s, 1 H), 4.33 (m, 1 H), 2.84 (dd, 1 H), 2.60−2.77

(m, 3 H), 2.31 (m, 4 H); MS (ES-) 564. Anal. ($C_{24}H_{27}N_3O_{11}S$·1.34H_2O) C, H, N.

2-(4-{(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-[(4-hydroxyphenethyl)amino]-3-oxopropyl}phenoxy)malonic acid (97) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.06 (d, 1 H), 7.90 (t, 1 H), 7.09 (d, 2 H), 6.95 (d, 2 H), 6.79 (d, 2 H), 6.64 (d, 2 H), 5.27 (s, 1 H), 4.31 (m, 1 H), 3.15 (m, 2 H), 2.85 (dd, 1 H), 2.63 (m, 1 H), 2.52 (m, 2 H), 2.33 (m, 4 H); MS (ES−) 501; HRMS (FAB) calcd for $C_{24}H_{26}N_2O_{10}$ + H 503.1665; found 503.1656.

2-(4-{(2*S*)-3-[(4-Carboxybenzyl)amino]-2-[(3-carboxypropanoyl)amino]-3-oxopropyl}phenoxy)malonic acid (98) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.49 (t, 1 H), 8.20 (d, 1 H), 7.85 (d, 2 H), 7.27 (d, 2 H), 7.13 (d, 2 H), 6.80 (d, 2 H), 5.28 (s, 1 H), 4.45 (m, 1 H), 4.31 (m, 2 H), 2.95 (dd, 1 H), 2.71 (dd, 1 H), 2.34 (m, 4 H); MS (FAB) *m*/*z* (rel intensity) 517 (MH^+, 39), 517 (39), 391 (76), 149 (99), 113 (30), 71 (39), 69 (37), 57 (75), 55 (49), 43 (48), 41 (37); HRMS (FAB) calcd for $C_{24}H_{24}N_2O_{11}$ + H 517.1458; found 517.1451. Anal. ($C_{24}H_{24}N_2O_{11}$·0.64H_2O) C, H; N: calcd, 5.31; found, 4.82.

2-[4-((2*S*)-2-[(4-Hydroxy-4-oxobutanoyl)amino]-3-oxo-3-{[4-(trifluoromethyl)benzyl]amino}propyl)phenoxy]-malonic acid (99) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.50 (t, 1 H), 8.18 (d, 1 H), 7.62 (d, 2 H), 7.31 (d, 2 H), 7.12 (d, 2 H), 6.80 (d, 2 H), 5.27 (s, 1 H), 4.43 (m, 1 H), 7.32 (d, 2 H), 3.91 (dd, 1 H), 3.71 (dd, 1 H), 2.33 (m, 4 H); MS (ES−) 539. Anal. ($C_{24}H_{23}F_3N_2O_9$·0.62H_2O) C, H, N.

2-(4-{(2*S*)-3-[(1,3-Benzodioxol-5-ylmethyl)amino]-2-[(3-carboxypropanoyl)amino]-3-oxopropyl}phenoxy)-malonic acid (100) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.32 (t, 1 H), 8.11 (d, 1 H), 7.10 (d, 2 H), 6.78 (m, 4 H), 5.59 (d, 1 H), 5.95 (s, 2 H), 5.23 (s, 1 H), 4.40 (m, 1 H), 4.14 (d, 2 H), 3.92 (dd, 1 H), 2.70 (m, 1 H), 2.30 (m, 4 H); MS (ES−) 515. Anal.($C_{24}H_{24}N_2O_{11}$·2.3H_2O) C, H, N.

2-(4-{(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-[(2-phenoxyethyl)amino]propyl}phenoxy)malonic acid (101) was prepared by the general procedure described for the preparation of **30** from **28b**: ^{1}H NMR (DMSO) δ 8.15 (t, 1 H), 8.09 (d, 1 H), 7.27 (t, 2 H), 7.10 (d, 2 H), 6.92 (m, 3 H), 7.76 (d, 2 H), 5.23 (s, 1 H), 4.40 (m, 1 H), 3.90 (t, 2 H), 2.87 (dd, 1 H), 2.65 (dd, 1 H), 2.30 (m, 4 H); MS (FAB) *m*/*z* (rel intensity) 503 (MH^+, 13), 392 (18), 391 (62), 149 (99), 113 (30), 71 (33), 69 (18), 57 (49), 55 (22), 43 (30), 41 (20); HRMS (FAB) calcd for $C_{24}H_{26}N_2O_{10}$ + H 503.1665, found 503.1656. Anal. ($C_{24}H_{26}N_2O_{10}$) C; H: calcd, 5.22; found, 5.79; N: calcd, 5.57; found, 4.96.

2-[4-((2*S*)-2-[(3-Carboxypropanoyl)amino]-3-oxo-3-{[2-(1-piperidinyl)ethyl]amino}propyl)phenoxy]malonic acid (102) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (DMSO) δ 1.47 (brs, 4 H), 1.66 (brs, 4 H), 2.33 (brs, 4 H), 2.76 (m, 4 H), 3.05 (brs, 4 H), 4.30 (m, 1 H), 5.04 (s, 1 H), 6.64 (d, 2 H), 7.01 (d, 2 H), 8.02 (m, 1 H), 8.16 (m, 1 H)(3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 494 (MH^+, 96), 495 (29), 494 (96), 450 (67), 392 (37), 133 (32), 98 (99), 71 (27), 57 (39), 45 (34), 43 (29). Anal. ($C_{23}H_{31}N_3O_9$·1.42H_2O) C, H, N.

2-[4-((2*S*)-2-[(3-Carboxypropanoyl)amino]-3-{[3-(4-morpholinyl)propyl]amino}-3-oxopropyl)phenoxy]malonic acid (103) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (DMSO) δ 2.32 (m, 6 H), 2.50−2.80 (m, 6 H), 2.97 (m, 4 H), 3.76 (m, 4 H), 4.31 (m, 1 H), 5.10 (s, 1 H), 6.68 (d, 2 H), 7.00 (d, 2 H), 7.79 (m, 1 H), 8.05 (m, 1 H) (3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 510 (MH^+, 99), 511 (21), 510 (99), 466 (34), 463 (30), 308 (29), 241 (24), 177 (23), 100 (40), 57 (23), 39 (21). Anal. ($C_{23}H_{31}N_3O_{10}$·2.0H_2O) C, H, N.

2-[4-((2*S*)-2-[(3-Carboxypropanoyl)amino]-3-{[3-(1H-imidazol-1-yl)propyl]amino}-3-oxopropyl)phenoxy]malonic acid (104) was prepared by the general procedure described for the preparation of **30** from **28a**: ^{1}H NMR (DMSO/DCl) δ 0.93 (t, 2 H), 1.80−1.93 (m, 3 H), 2.28 (m, 3 H), 3.03 (m, 4 H), 4.02 (m, 1 H), 5.00 (s, 1 H), 6.78 (d, 2 H), 7.12 (d, 2 H), 7.30 (s, 1 H), 7.63 (s, 1 H), 7.71 (s, 1 H) (NH's, 3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 491 (MH^+, 1), 505 (3), 383 (8), 357 (4), 236 (11), 222 (23), 174 (76), 127 (17), 124 (7), 118 (8), 107 (99).

2-(4-{(2*S*)-3-{[(1*S*)-1-(Aminocarbonyl)pentyl]amino}-2-[(3-carboxypropanoyl)amino]-3-oxopropyl}phenoxy)-malonic acid (105) was prepared by the general procedure described for the preparation of **30** from **28b**, using (*S*)-norleucine amide as the amine component: ^{1}H NMR (DMSO) δ 0.83 (t, 3 H), 1.19 (m, 4 H), 1.53 (m, 1 H), 1.68 (m, 1 H), 2.30 (m, 4 H), 2.71 (m, 1 H), 2.95 (m, 1 H), 4.02 (m, 1 H), 4.40 (m, 1 H), 5.30 (s, 1 H), 6.79 (d, 2 H), 6.99 (s, 1 H), 7.09 (s, 1 H), 7.13 (d, 2 H), 7.80 (d, 1 H), 8.10 (d, 1 H) (3-CO_2H absent); HRMS (FAB) calcd for $C_{22}H_{29}N_3O_{10}$ + H 496.1931, found 496.1929. Anal.($C_{22}H_{29}N_3O_{10}$·1.5H_2O) C, H, N.

2-[4-((2*S*)-2-[(3-Carboxypropanoyl)amino]-3-{[(1*S*)-1-(hydroxymethyl)-3-methylbutyl]amino}-3-oxopropyl)-phenoxy]malonic acid (106) was prepared by the general procedure described for the preparation of **30** from **28b**, using (*S*)-leucinol as the amine component: ^{1}H NMR (DMSO-d_6) δ 7.99 (d, 1 H), 7.43 (d, 1 H), 7.11 (d, 2 H), 6.78 (d, 2 H), 5.25 (s, 1 H), 4.35 (m, 1 H), 7.72 (m, 1 H), 3.25 (dd, 1 H), 3.12 (dd, 1 H), 2.87 (dd, 1 H), 2.65 (dd, 1 H), 2.31 (m, 4 H), 1.52 (m, 1 H), 1.26 (m, 2 H), 0.82 (q, 6 H); MS (FAB) *m*/*z* (rel intensity) 483 (MH^+, 91), 484 (22), 483 (91), 139 (25), 123 (18), 118 (99), 105 (17), 103 (15), 91 (18), 86 (16), 55 (15); HRMS (FAB) calcd for $C_{22}H_{30}N_2O_{10}$ + H 483.1978, found 483.199. Anal. ($C_{22}H_{30}N_2O_{10}$·1.4H_2O) C, H, N.

2-{4-[(2*S*)-2-({(2*S*)-4-Carboxy-2-[(3-carboxypropanoyl)-amino]butanoyl}amino)-3-oxo-3-(pentylamino)propyl]-phenoxy}malonic acid (107) was prepared by procedures analogous to those described for the preparation of **34** from **12a** (Scheme 5): ^{1}H NMR (DMSO) δ 0.82 (t, 3 H), 1.20 (m, 3 H), 1.32 (m, 3 H), 1.55−1.90 (m, 2H), 2.14 (t, 2 H), 2.38 (m, 4 H), 2.72 (m, 2 H), 2.92 (m, 4 H), 4.11 (m, 1 H), 4.30 (m, 1 H), 5.21 (s, 1 H), 6.77 (d, 2 H), 7.67 (t, 1 H), 7.77 (d, 1 H), 8.08 (d, 1 H) (4-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 582 (MH^+, 51), 582 (51), 238 (41), 133 (42), 102 (81), 102 (99), 88 (92), 84 (64), 43 (39), 39 (75), 23 (78). Anal. ($C_{26}H_{35}N_3O_{12}$·1.37H_2O) C, H, N.

2-{4-[(2*S*)-2-({(2*S*)-2-[(*tert*-butoxycarbonyl)amino]-4-carboxybutanoyl}amino)-3-oxo-3-(pentylamino)propyl]-phenoxy}malonic acid (108) was prepared by procedures analogous to those described for the preparation of **32** from **12a** (Scheme 5): ^{1}H NMR (DMSO) δ 0.81 (t, 3 H), 1.18 (m, 15 H), 1.71 (m, 2 H), 2.12 (t, 2 H), 2.96−3.10 (m, 4 H), 3.82 (m, 1 H), 4.38 (m, 1 H), 5.19 (s, 1 H), 6.76 (d, 2 H), 6.96 (d, 1 H), 7.08 (d, 2 H), 7.77 (d, 1 H), 7.84 (brt, 1 H) (3-CO_2H absent); MS (FAB) *m*/*z* (rel intensity) 582 (MH^+, 2), 133 (19), 102 (25), 88 (54), 84 (25), 57 (99), 43 (24), 41 (43), 39 (16), 29 (40), 23 (52). Anal. ($C_{27}H_{39}N_3O_{11}$·1.22H_2O) C, H, N.

2-{4-[(2*S*)-2-[((2*S*)-2-{[(Benzyloxy)carbonyl]amino}-3-carboxypropanoyl)amino]-3-oxo-3-(pentylamino)propyl]-phenoxy}malonic acid (109) was prepared from **12a** by procedures analogous to those described for the preparation of **32** (Scheme 5), except that Cbz-Asp(OBn)OSu was used in place of Boc-Asp(OBn)OSu and saponification with LiOH was used in the final step instead of hydrogenation: ^{1}H NMR (DMSO) δ 8.10 (d, 1 H), 7.80 (t, 1 H), 7.45 (d, 1 H), 7.31 (m, 5 H), 7.00 (d, 2 H), 6.78 (d, 2 H), 5.24 (s, 1 H), 5.00 (s, 2 H), 4.32 (m, 1 H), 2.80−3.00 (m, 3 H), 2.55−2.70 (m, 3 H), 1.10−1.35 (m, 6 H), 0.82 (t, 3 H); MS (FAB) *m*/*z* (rel intensity) 602 (MH^+, 37), 602 (37), 392 (17), 391 (54), 149 (63), 113 (18), 91 (99), 74 (34), 71 (22), 57 (29), 43 (22); HRMS (FAB) calcd for $C_{29}H_{35}N_3O_{11}$ + H 602.2349, found 602.2363; Anal. ($C_{29}H_{35}N_3O_{11}$·H_2O) C, H, N.

2-{4-[(2*S*)-2-({(2*R*)-3-(4-Benzoylphenyl)-2-[(3-carboxypropanoyl)amino]propanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (110) was prepared by procedures identical to those described for the preparation of **38** from **12b**, starting with D-Boc-Bpa-OH: ^{1}H NMR (DMSO-d_6) δ 8.38 (d, 1 H), 8.10 (d, 1 H), 7.90 (t, 1 H), 7.67 (m, 3 H), 7.55 (m, 4 H), 7.25 (d, 2 H), 7.25 (d, 2 H), 7.79

(d, 2 H), 5.21 (s, 1 H), 4.52 (m, 1 H), 4.38 (m, 1 H), 3.00 (m, 2 H), 2.75−2.90 (m, 2 H), 2.60 (dd, 2 H), 2.27 (m, 4 H), 1.21 (m, 6 H), 0.83 (t, 3 H); MS (FAB) *m/z* (rel intensity) 704 (MH^+, 99), 705 (42), 704 (99), 353 (20), 238 (35), 224 (70), 219 (27), 194 (19), 107 (28), 105 (34), 88 (49); HRMS (FAB) calcd for $C_{37}H_{41}N_3O_{11}$ + H 704.2819, found 704.2822. Anal. Calcd for $C_{37}H_{41}N_3O_{11}$: C, 63.15; H, 5.87; N, 5.97. Found: C, 63.69; H, 6.36; N, 5.24.

2-{4-[(2*S*)-2-({(2*S*)-3-[4-(Benzyloxy)phenyl]-2-[(3-carboxypropanoyl)amino]propanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (111) was prepared by procedures analogous to those described for the preparation of **38** from **12b** (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 8.05 (d, 1 H), 7.95 (d, 1 H), 7.66 (t, 1 H), 7.36 (m, 5 H), 7.09 (t, 4 H), 6.82 (dd, 4 H), 5.25 (s, 1 H), 5.02 (s, 2 H), 4.34 (m, 2 H), 2.97 (m, 2 H), 2.95−2.90 (m, 2 H), 2.90 (dd, 1 H), 2.61 (dd, 1 H), 2.30 (m, 4 H), 1.33−1.13 (m, 6 H), 0.82 (t, 3 H); MS (FAB) *m/z* (rel intensity) 706 (MH^+, 36), 707 (15), 706 (36), 353 (15), 238 (13), 226 (28), 91 (99), 88 (16), 57 (20), 55 (15), 43 (20); HRMS (FAB) calcd for $C_{37}H_{43}N_3O_{11}$ + H 706.2975, found 706.2986. Anal.($C_{37}H_{43}N_3O_{11}$·0.80H_2O) C, H, N.

2-{4-[(2*S*)-2-[((2*S*)-2-[(3-Carboxypropanoyl)amino]-3-{4-[(2,6-dichlorobenzyl)oxy]phenyl}propanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (112) was prepared by procedures analogous to those described for the preparation of **38** from **12b** (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1 H), 7.95 (d, 1 H), 7.67 (t, 1 H), 7.54 (m, 2 H), 7.45 (dd, 1 H), 7.11 (m, 4 H), 6.91 (d, 2 H), 6.81 (d, 2 H), 5.26 (s, 1 H), 5.15 (s, 2 H), 4.35 (m, 2 H), 2.98 (m, 2 H), 2.85 (m, 2 H), 2.73 (dd, 1 H), 2.62 (dd, 1 H), 2.33 (m, 4 H), 1.33−1.12 (m, 6 H), 0.81 (t, 3 H); MS (FAB) *m/z* (rel intensity) 774 (MH^+, 99), 776 (70), 775 (53), 774 (99), 391 (97), 294 (48), 161 (54), 159 (80), 149 (63), 136 (39), 88 (46); HRMS (FAB) calcd for $C_{37}H_{41}Cl_2N_3O_{11}$ + H 774.2196, found 774.2224. Anal. ($C_{37}H_{41}Cl_2N_3O_{11}$·0.36$H_2O$) C, H, N.

2-{4-[(2*S*)-2-{[(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-(4-methoxyphenyl)propanoyl]amino}-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (113) was prepared by procedures analogous to those described for the preparation of **38** from **12b** (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 8.02 (d, 1 H), 7.90 (d, 1 H), 7.64 (t, 1 H), 7.09 (m, 4 H), 6.78 (m, 4 H), 5.25 (s, 1 H), 4.35 (m, 2 H), 3.68 (s, 3 H), 2.98 (m, 2 H), 2.9−2.70 (m, 3 H), 2.60 (dd, 1 H), 2.30 (m, 4 H), 1.35−1.10 (m, 6 H), 0.82 (t, 3 H); MS (FAB) *m/z* (rel intensity) 630 (MH^+, 81), 631 (28), 630 (81), 353 (25), 250 (25), 238 (23), 177 (30), 161 (28), 150 (99), 121 (44), 88 (39); HRMS (FAB) calcd for $C_{31}H_{39}N_3O_{11}$ + H 630.2662, found 630.2661. Anal. ($C_{31}H_{39}N_3O_{11}$·0.54H_2O) C, H, N.

2-{4-[(2*S*)-2-({(2*S*)-2-[(3-Carboxypropanoyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (114) was prepared by procedures analogous to those described for the preparation of **38** from 12b (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 8.10 (d, 1 H), 7.96 (d, 1 H), 7.69 (t, 1 H), 7.15 (m, 7 H), 6.80 (d, 2 H), 5.26 (s, 1 H), 4.35 (m, 2 H), 2.92 (m, 4 H), 2.71 (m, 2 H), 2.80 (m, 4 H), 1.25 (m, 6 H), 0.82 (t, 3 H); MS (FAB) *m/z* (rel intensity) 600 (MH^+, 35), 600 (35), 155 (22), 149 (50), 136 (20), 120 (99), 88 (27), 73 (32), 71 (23), 57 (34), 43 (26); HRMS (FAB) calcd for $C_{30}H_{37}N_3O_{10}$ + H 600.2557, found 600.2579. Anal.($C_{30}H_{37}N_3O_{10}$·0.51H_2O) C, H, N.

2-{4-[(2*S*)-2-[((2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-{4-[(2,6-dichlorobenzyl)oxy]phenyl}propanoyl)amino]-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (115) was prepared by procedures analogous to those described for the preparation of **37** from **12b** (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 7.87 (m, 2 H), 7.53 (m, 2 H), 7.46 (m, 1 H), 7.11 (d, 2 H), 6.91 (d, 2 H), 6.89 (m, 1 H), 6.80 (d, 2 H), 5.23 (s, 1 H), 5.15 (s, 2 H), 4.40 (m, 1 H), 4.05 (m, 1 H), 2.97 (m, 2 H), 2.78 (m, 3 H), 2.60 (m, 1 H), 1.35−1.14 (m, 15 H), 0.81 (t, 3 H); MS (FAB) *m/z* (rel intensity) 774 (MH^+, 8), 296 (28), 294 (41), 238 (39), 194 (31), 161 (52), 159 (81), 136 (31), 88 (56), 57 (99), 41 (24); HRMS (FAB) calcd for $C_{38}H_{45}Cl_2N_3O_{10}$ + H 774.2560, found 774.2557. Anal. ($C_{38}H_{45}Cl_2N_3O_{10}$·0.92$H_2O$) C, H, N.

2-{4-[(2*S*)-2-{[(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-(4-methoxyphenyl)propanoyl]amino}-3-oxo-3-(pentylamino)-propyl]phenoxy}malonic acid (116) was prepared by procedures analogous to those described for the preparation of **37** from **12b** (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 7.85 (m, 2 H), 7.09 (m, 4 H), 6.78 (m, 5 H), 5.22 (s, 1 H), 4.40 (m, 1 H), 4.02 (m, 1 H), 3.68 (s, 3 H), 2.97 (m, 2 H), 3.90−3.70 (m, 3 H), 2.60 (m, 1 H), 1.34−1.12 (m, 15 H), 0.82 (t, 3 H); MS (FAB) *m/z* (rel intensity) 630 (MH^+, 11), 574 (24), 238 (29), 194 (31), 177 (34), 161 (29), 150 (99), 136 (24), 121 (65), 88 (64), 57 (87); HRMS (FAB) calcd for $C_{32}H_{43}N_3O_{10}$ + H 630.3026, found 630.3015. Anal. ($C_{32}H_{43}N_3O_{10}$·1.64H_2O) C, H, N.

2-{4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]phenoxy}malonic acid (117) was prepared by procedures analogous to those described for the preparation of **37** from **12b** (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 7.87 (m, 2 H), 7.17 (m, 5 H), 6.90 (d, 1 H), 6.80 (d, 2 H), 5.22 (s, 1 H), 4.41 (m, 1 H), 4.10 (m, 1 H), 2.97 (m, 2 H), 2.83 (m, 2 H), 2.70−2.50 (m, 2 H), 1.34−1.12 (m, 15 H), 0.82 (t, J = 3 Hz, H); MS (FAB) *m/z* (rel intensity) 600 (MH^+, 27), 600 (27), 544 (33), 238 (22), 136 (22), 133 (22), 120 (99), 88 (61), 57 (87), 43 (18), 41 (20); HRMS (FAB) calcd for $C_{31}H_{41}N_3O_9$ + H 600.2921, found 600.2923. Anal. ($C_{31}H_{41}N_3O_9$·0.96H_2O) C, H, N.

2-{4-[(2*S*)-2-({(2*S*)-2-[(*tert*-Butoxycarbonyl)amino]-propanoyl}amino)-3-oxo-3-(pentylamino)propyl]-phenoxy}malonic acid (118) was prepared by procedures analogous to those described for the preparation of **37** from **12b** (Scheme 7): ^{1}H NMR (DMSO-d_6) δ 7.80 (m, 1 H), 7.69 (d, 1 H), 7.08 (d, 2 H), 6.95 (d, 1 H), 6.77 (d, 2 H), 5.22 (s, 1 H), 4.35 (m, 1 H), 3.80 (m, 1 H), 2.95 (m, 2 H), 2.95−2.85 (m, 1 H), 2.75 (m, 1 H), 1.35−1.15 (m, 15 H), 1.05 (d, 3 H), 0.82 (t, 3 H); MS (FAB) *m/z* (rel intensity) 524 (MH^+, 13), 468 (39), 238 (25), 136 (21), 133 (20), 88 (99), 86 (19), 57 (71), 44 (58), 41 (19), 29 (18); HRMS (FAB) calcd for $C_{25}H_{37}N_3O_9$ + H 524.2607, found 524.2612. Anal. Calcd for $C_{25}H_{37}N_3O_9$·2.22H_2O) C, H, N.

5-[(2*S*)-2-({(2*R*)-2-[(*tert*-Butoxycarbonyl)amino]-3-phenylpropanoyl}amino)-3-oxo-3-(pentylamino)propyl]-2-(carboxymethoxy)benzoic acid (119) was prepared by procedures identical to those described for the preparation of **69** from **55**, using *N*-Boc-D-Phe-OH (Scheme 13): ^{1}H NMR (DMSO-d_6) δ 8.30 (d, J = 8 Hz, 1 H), 7.84 (bt, J = 6 Hz, 1 H), 7.58 (bs, 1 H), 7.27 (bd, J = 8 Hz, 1 H), 7.15 (m, 5 H), 6.88 (d, J = 9 Hz, 1 H), 6.79 (d, J = 8 Hz, 1 H), 4.66 (s, 2 H), 4.38 (m, 1 H), 4.11 (m, 1 H), 2.5−3.1 (m, 6 H), 1.1−1.4 (m, 6 H), 1.27 (s, 9 H), 0.83 (t, J = 7 Hz, 3 H); MS (ESI−) for $C_{31}H_{41}N_3O_9$ *m/z* 598.4 ($M - H)^-$; HRMS (FAB) calcd for $C_{31}H_{41}N_3O_9$ + H 600.2921, found 600.2930.

Assays for PTP Activity. PTP catalytic activities were routinely measured as rates of hydrolysis of *p*-nitrophenyl phosphate (pNPP) in a 96-well microtiter plate format. Sources of the PTP enzymes used as well as details of the PTP assays are described elsewhere.[19] Inhibitors were added to PTP assay mixtures as solutions in DMSO at 100 times the final concentration. For determinations of inhibitor kinetics, plots of V versus S at various concentrations of inhibitor were generated and K_i values were computed using the direct linear method of Cornish−Bowden (Enzpack3 software, Biosoft, Cambridge, UK).

Measurement of Cellular Insulin Response. For the evaluation of PTP1B inhibitors as enhancers of insulin action, we measured rates of uptake of 2-deoxyglucose by L6 myocytes in the absence and presence of insulin at a concentration (10 nM) that supported half-maximal uptake.[19] Data are expressed as percent basal (unstimulated) in the absence of compound and as percent insulin-stimulated, i.e.,

$$[(\text{uptake with insulin plus test cpd}) - (\text{uptake without insulin plus test cpd})]/[(\text{uptake with insulin no test cpd}) - (\text{uptake without insulin no test cpd})] \times 100\%$$

A value of ≥120% was scored as active.

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Supporting Information Available: Crystallography materials and methods for PTP1B−compound **69** complex. This material is available free of charge via the Internet at http://pubs.acs.org.

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