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Design, Synthesis and Evaluation of D-Homophenylalanyl Epoxysuccinate Inhibitors of the Trypanosomal Cysteine Protease Cruzain

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Abstract—The binding modes of E-64c to papain combined with molecular modeling and ligand design using the crystal structure of cruzain have been used to develop new, potent D-Homophenylalanyl epoxysuccinate inhibitors of cruzain, the major cysteine protease of *Trypanosoma cruzi*. The most potent inhibitor **47** contains an *O*-benzyl hydroxamate unit and is highly specific for cruzain relative to other cysteine proteases tested.

Introduction

Cysteine proteases regulate a broad range of biological functions and are emerging as attractive targets for drug design in a variety of pathological processes including arthritis, osteoporosis, Alzheimer's disease, cancer cell invasion and apoptosis.[1–3] Cysteine proteases also play crucial roles in the life cycle of parasites that cause infections such as malaria, leishmaniasis and Chagas' disease.[4–6] Chagas' disease, or American trypanosomiasis, is transmitted to humans by blood sucking triatomine vectors infected with *Trypanosoma cruzi* and is characterized by chronic digestive lesions[7] and cardiopathy.[8] At least 16–18 million people are infected in Latin America where the disease is endemic, resulting in more than 50,000 deaths each year.[9,10] The traditional drugs used to treat Chagas' disease are effective only in the early stages of the infection but cause serious side effects.[11,12] There are no drugs for treatment of the chronic infection. Recently McKerrow and Urbina have independently described the first successful treatments of Chagas' disease in mouse models.[13,14] Cruzain, a cysteine protease of the papain family, is the major cysteine protease of *T. cruzi* and is essential for replication and differentiation of the intracellular parasite.[13,15] Cruzain thus represents an attractive chemotherapeutic target for treatment of Chagas' disease. Other promising therapeutic approaches under development target trypanothione reductase[16–18] and sterol C-14α-demethylase.[14,19,20]

Malaria is one of the most important infectious diseases worldwide. Over one million people die each year from infections caused by *Plasmodium falciparum*, the most virulent human malaria parasite.[21] The problem is exacerbated by the increasing resistance of malaria parasites to drugs. A new strategy for malaria chemotherapy targets the inhibition of hemoglobin degradation.[22] *P. falciparum*uses cysteine and aspartic proteases to degrade hemoglobin from which it obtains the amino acids necessary for protein synthesis. One of the hemoglobinases involved is falcipain,[23] a cysteine protease of the papain family whose inhibition results in arrest of parasite development.[24,25] Falcipain inhibitors are therefore of considerable interest as potential anti-malarial agents.[26]

Leishmaniasis is a third, significant parasitic disease with a wide range of clinical courses that include skin and visceral lesions (kala azar) that may be fatal if the disease is not treated. Leishmaniasis affects 12 million people in over 88 countries.[27] The cathepsin B-like protease from *Leishmania major* is yet another cysteine protease of the papain family that has been identified as a potential target for leishmaniasis chemotherapy.[28,29]

In connection with ongoing efforts to develop potent and selective inhibitors of cruzain[30–32] we became interested in a class of naturally occurring peptidyl epoxysuccinate derivatives of which E-64 (**1**),[33–35] cathestatin C (**2**)[36,37] and circinamide (**3**)[38] are representative examples. Peptidyl epoxysuccinates are potent and selective irreversible

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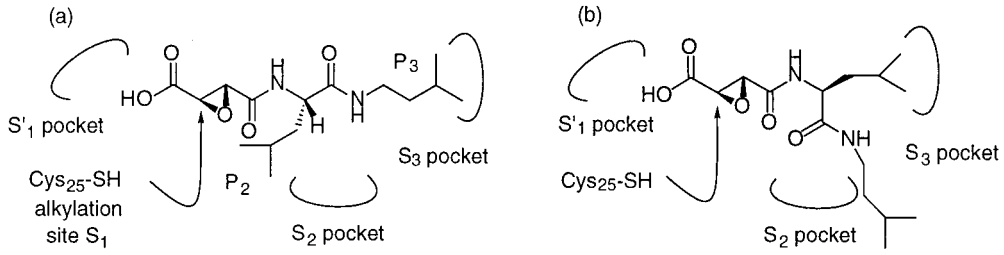
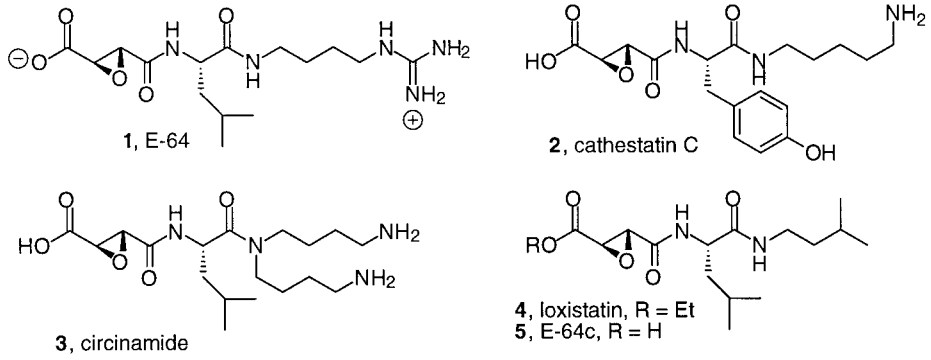


Scheme 1. Alternative binding modes of E-64c to papain. (a) The L-leucyl side chain is located at the edge of the hydrophobic S_2 pocket of papain. (b) The isopentylamine is located at the edge of the S_2 pocket in the second binding mode. The terminology used is that of Schechter and Berger[54] who described seven binding sites, S_4–S'_3, in papain.



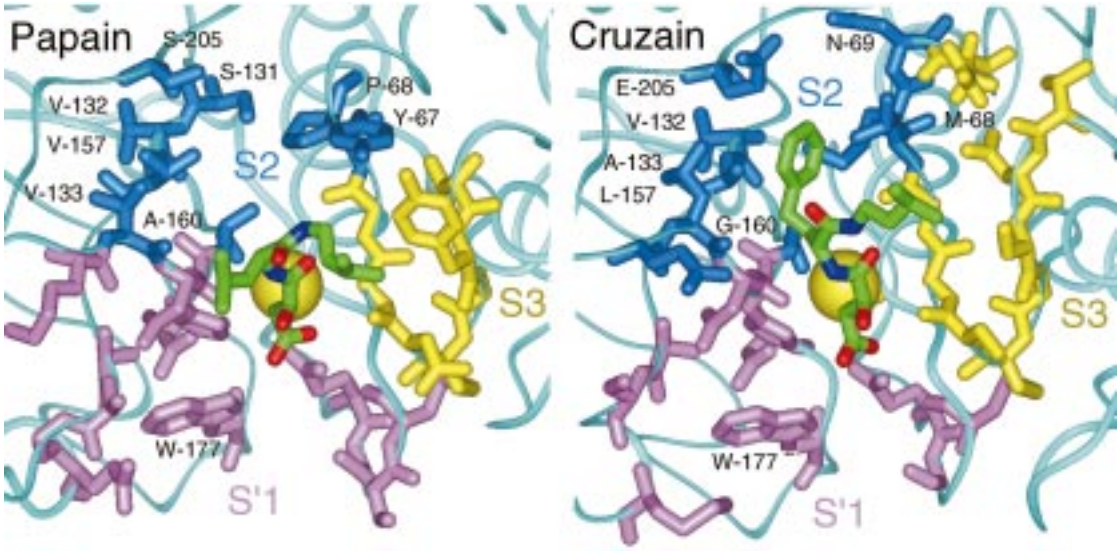
inhibitors of cysteine proteases. E-64 does not react with thiols in solution and does not inhibit aspartyl or serine proteases. These attributes have stimulated considerable interest in the development of analogs for therapeutic purposes. Loxistatin (**4**),[39,40] the ethyl ester prodrug of E-64c (**5**), was developed for treatment of muscular dystrophy and was taken into clinical trials in Japan.[41] More recently, selective and potent inhibitors of cathepsins B[42–46] and L[47,48] have been developed based on the epoxysuccinyl motif.

E-64 inhibits cysteine proteases by irreversible alkylation of the active site cysteine residue. The regiochemistry of the inhibition reaction of papain with E-64 was determined by Rich[49] who established that substitution of the epoxide by the cysteine -SH unit occurs at C(2), adjacent to the carboxylic acid unit (which is fully deprotonated and negatively charged at physiological pH). This conclusion has been verified by X-ray crystal structures of the E-64·papain and E-64c·papain complexes.[50–52] E-64c binds to papain in two alternate modes (see Scheme 1) in which the leucyl side chain and the isopentylamine interchange binding in the S_2 and S_3 sites.[51,52] Inspection of these structures reveals that the hydrophobic S_2 binding pocket, which is regarded as the most important element of substrate specificity for members of the papain family of cysteine proteases,[53,54] is not optimally filled in either mode. In structure (a), the leucyl side chain is bound to the rim of the S_2 pocket, whereas in structure (b) the isoamyl chain interacts with the rim of the S_2 pocket (see Scheme 1). Armed with these insights, we embarked on a program designed to optimize the interactions of epoxysuccinyl inhibitors with the S_2 and S_3 binding pockets of cruzain.[55]

Results and Discussion

Examination of the first crystal structure of the E-64c·papain complex[51] (Scheme 1a) reveals that the α-hydrogen, and not the side chain of the L-leucyl residue, is oriented directly into the hydrophobic S_2 pocket. We envisioned that simple inversion of the amino acid configuration from L to D would place the leucyl side chain within the S_2 pocket, thus improving enzyme-inhibitor interactions. The S_2 site of papain is regarded as the most important element of substrate specificity, and is highly selective for hydrophobic L-amino acids.[52,53] Consequently, the use of D-amino acids in design of inhibitors of papain-family cysteine proteases has been rather limited, with most of these studies involving substrate-like peptidomimetics. Several different classes of inhibitors containing a D-amino acid at P_2 have been evaluated vs papain and all proved to be inferior to the peptidyl inhibitors containing the corresponding L-amino acid.[56–58] Lalmanach and coworkers have recently revisited the S_2 specificity of papain with fluorogenic substrates that contain structural analogs of phenylalanine as P_2 residues.[59] These investigators found that papain can accommodate D-Phe and L-hPhe as P_2 residues but the kinetic data shows a significant preference for L-Phe>D-Phe>L-hPhe. In a recent report Chatterjee and coworkers described the first successful design of potent, D-amino acid containing inhibitors of calpain I.[60]

Nevertheless, in spite of the unfavorable literature precedent, we decided it was worthwhile to explore a new series of inhibitors based on the E-64c motif, containing D-amino acid residues in place of L-Leu residue of the parent compound. The question was how best to identify



Scheme 2. Left panel: Crystal structure of E64-c bound to papain. The leucyl side chain of the inhibitor is outside of the S2 hydrophobic pocket. Right panel: Model of the D-hPhe analog **11c** bound to cruzain, with the phenyl group of the h-Phe side chain optimally occupying the S2 hydrophobic pocket. The binding pockets are indicated by color codes: S′1 (pink), S2 (blue) and S3 (yellow). The nucleophilic sulfur atom of the active site Cys-25 is indicated as a yellow sphere. The atoms of the inhibitors are color coded as follows: C (green), O (red), and N (blue).

suitable lead structures? We turned to structure-based design paradigms to aid this phase of the work.

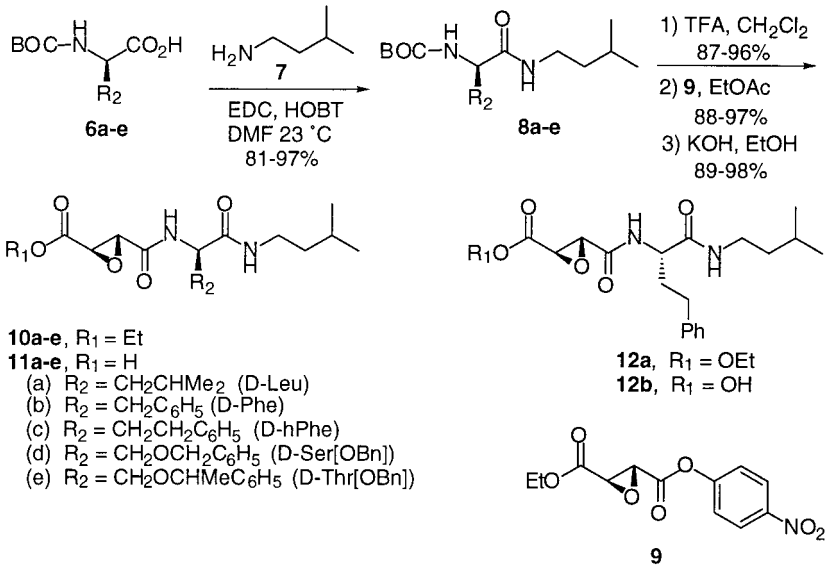
We transferred the E-64c coordinates from the papain·E-64c structure to the crystal structure of cruzain.[61] Using the Insight II software package[62] we exchanged the stereochemistry of the aminoacid at P_2 in the inhibitor from L-Leu to D-Leu. Minimization of the resulting (bound) peptidyl epoxysuccinyl unit in the active site suggested that the cruzain S_2 pocket could accept further structural variation of the amino acid side chain. The ligand design software package LUDI[63] was used then to identify an appropriate P_2 group for our template. LUDI uses the crystal structure of a protein to identify possible binding sites at positions where hydrogen bonds can be formed with the enzyme, or hydrophobic pockets can be occupied by lipophilic groups. LUDI then selects fragments from a library that fit into the interaction sites and finally prioritizes the selected fragments by taking into account hydrogen bonds, ionic interactions, lipophilic protein-ligand contact surface and number of rotatable bonds in the ligand. Two major strategies exist for this fragment based approach to ligand design. One can obtain several independent fragments and then connect them by means of an appropiate template in one molecule or, alternatively, one fragment can be positioned initially—in our case the epoxysuccinyl moiety—and then append additional fragments. We have used this latter linking mode. Accordingly, the isopropyl group of the L-leucyl side chain of the inhibitor was deleted and then the link mode of LUDI was used to identify the benzyl unit as the highest scoring fragment at the S_2 hydrophobic pocket of cruzain. (Other fragments retrieved were, in order of priority, isopropyl and methyl). Thus, this analysis led to the prediction that the unnatural amino acid D-homophenylalanine (D-hPhe) would make an excellent match to the steric and hydrophobic requirements of the cruzain S_2 hydrophobic site (Scheme 2).

We therefore set out to prepare and test a new series of D-amino acid containing inhibitors vs cruzain and other cysteine proteases. Initially, we utilized Yokoo's procedure[64] for the synthesis of **11c** and other E-64 analogs. Although the LUDI analysis predicted that **11c** containing a D-hPhe unit would be optimal, we elected to prepare a series of other E-64 analogs with D-amino acid units to explore the P_2/S_2 interactions with cruzain (Scheme 3).

The Yokoo synthesis involves the coupling of a BOC protected[65] amino acid (**6a**–**e**) with an amine (in this case isopentylamine, **7**) envisaged to serve as the P_3 substituent. Deprotection of the resulting peptidomimetic **8** followed by N-acylation with the *p*-nitrophenyl ester **9**[64] and ester hydrolysis completed the synthesis of inhibitors **11a**–**11e**. The *O*-benzyl serine and threonine derivatives **11d** and **11c** were included in this series in order to determine the optimal number of atoms separating the P_2 phenyl substituent from the peptide backbone in this inhibitor series.

Preliminary evaluation (IC_{50} determinations) of compounds **11a**–**e** as inhibitors of cruzain showed a peak of activity with the D-hPhe derivative **11c** (see Table 1) These results seemed to be consistent with our initial proposal based on the molecular modeling study, although these IC_{50} determinations also suggested that **11c** would be a much weaker inhibitor than E-64c (**5**) itself. However, IC_{50} values are virtually meaningless for irreversible, time dependent inhibitors since IC_{50} data are rigorously dependent on the conditions used for the assay (concentration, time, etc.), and one can reach misleading conclusions when comparing data obtained on different days with different batches of enzyme by different experimentalists. Nevertheless, we continue to use IC_{50} data as a rough measure of relative inhibitor activity, for a series of inhibitors studied under identical conditions with the same batch of enzyme.

To obtain a more reliable assessment of the potency of these



Scheme 3.

inhibitors, a select group was subjected to full kinetic analysis.[66,67] As a positive control, we included E-64c (**5**) and the L-hPhe epimer **12b**[55] of **11c** in this analysis. Dissapointingly, the results of these analyses indicate that the designed inhibitor, **11c**, is a relatively weak inhibitor of cruzain and the other cysteine proteases studied, as are the D-Leu and D-Phe containing inhibitors **11a** and **11b**. In contrast, both E-64c (**5**) and **12b** (the L-hPhe analog) are the best inhibitors of cruzain among this group. This behavior parallels what previously has been observed for E-64c and papain, where the L-amino acid derivatives are substantially better inhibitors that the D-amino acid analogs.[64]

These inhibitors were also tested against two other cysteine proteases: cathepsin B[67] and papain. The results (shown in Table 2) indicate that only **12b** has a modest selectivity for inhibition of cruzain compared to cathepsin B, and also that only **12b** shows any selectivity between cruzain and papain.

Table 1. IC_{50} data for inhibition of cruzain with inhibitors **5** and **11a–e**

Inhibitor	P_2 amino acid	IC_{50} vs Cruzain (μM)
5	L-Leu	0.01
11a	D-Leu	0.5
11b	D-Phe	1.0
11c	D-hPhe	0.15
11d	D-Ser (Obn)	0.5
11e	D-Thr (OBn)	0.5

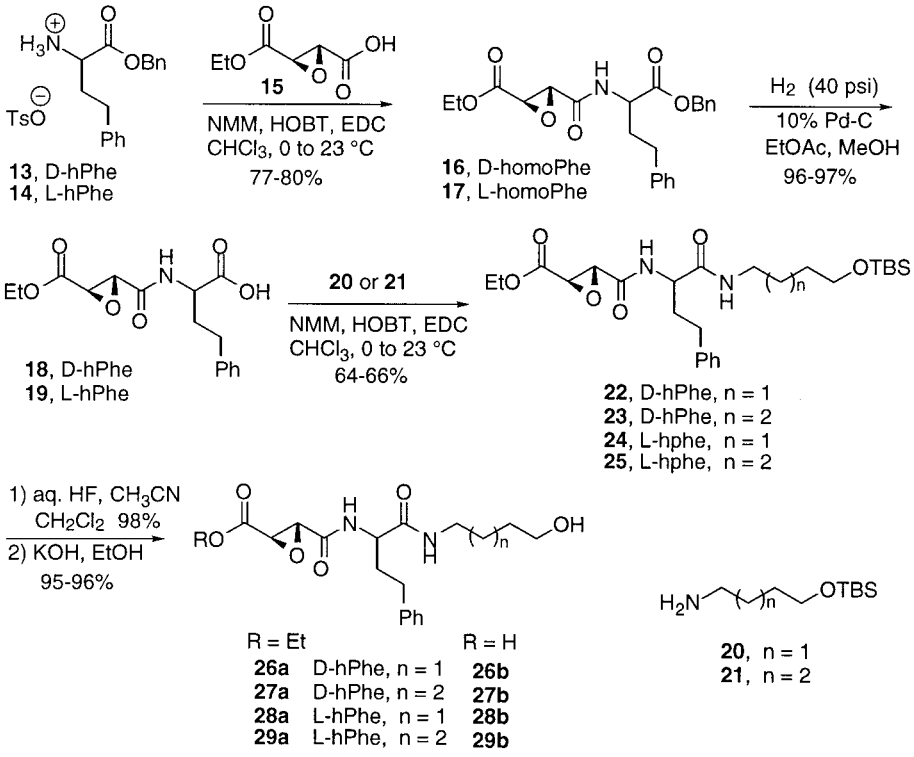
Table 2. Second order rate constants, k_{inact}/K ($s^{-1}\,M^{-1}$), of selected epoxysuccinate inhibitors determined against cruzain, bovine cathepsin B and papain. It was not possible to demonstrate saturation kinetics for **11c** against papain, owing to the range of inhibitor concentration that could be studied. Consequently, this rate constant is reported as k_{ass} (see Ref. [66]); nd=not determined

Inhibitor	P_2 Amino acid	vs cruzain k_{inact}/K_i	vs cath B k_{inact}/K_i	vs papain k_{inact}/K_i
5	L-Leu	70 600	52 300	200 000
11a	D-Leu	3800	900	23 700
11b	D-Phe	11 970	nd	4820
11c	D-hPhe	4584	3806	7900
12b	L-hPhe	65 000	7400	12 200

It should be noted that the data for inhibition of cruzain by **11c** in Table 2 are the results of repeated determinations. Initial kinetic analyses, the very first that we performed in this project, indicated that **11c** had a second order inhibiton rate constant vs cruzain of 77,000 $s^{-1}\,M^{-1}$. However, we have been unable to reproduce this rate constant in subsequent studies, and after multiple determinations we believe the value reported in Table 2 for **11c** to be correct. *Ironically, had the initial kinetic data indicated (correctly) that **11c** was a weak inhibitor, none of the studies subsequently described would have been performed. Ultimately, the fact that extremely potent D-amino acid inhibitors (e.g, **47**) derive from this research effort attests to the powerful role that serendipity can play in scientific research.*

A new synthesis of peptidyl epoxysuccinates to probe the S_3 site of cruzain

LUDI assisted ligand design[63] of the P_3 residue predicted that a lipophilic chain of 4–5 carbon atoms with a terminal polar group would interact with the Ser-61 residue at the rim of the S_3 hydrophobic pocket of cruzain. Similar P_3 residues are found in E-64 (**1**) and other natural product analogs.[33,36–38] Since the isopentyl amine chain of **5** was designed to avoid the bioavailability problems associated with the charged guanidinium moiety of **1**,[39,40] we focused on use of neutral alcohol derivatives at P_3. While Yokoo's synthetic method (Scheme 3) is extremely useful for varying the P_2 amino acid residue at the beginning of the synthesis, this route proved less convenient for synthesis of analogs designed to probe the requirements of the P_3/S_3 interactions, since the P_3 residue is introduced in the very first step. This prompted us to develop a new synthesis of peptidyl succinate derivatives that would permit the P_3 substituent to be introduced at the end of the sequence.[55] Thus, acylation of hPhe benzyl esters **13** and **14**[68,69] with the



Scheme 4.

epoxy acid **15**,[64] obtained from diethyl D-(−)-tartrate by the method of Mori and Iwasawa,[70] gave the amides **16**–**17**[55] in good yields. Hydrogenolysis of the benzyl ester afforded the acids **18** and **19**,[55] which were then coupled to the silyloxy amines **20** and **21**[71] to afford the corresponding peptidomimetics (**22**–**25**). Removal of the silyl protecting group and hydrolysis of the ethyl ester gave the desired epoxysuccinates **26b**–**29b** (Scheme 4).

Inhibitors **26b**–**29b** were submitted to kinetic assays with cruzain, cathepsin B and papain (Table 3). Disappointingly, the D-hPhe inhibitor analogs **26b** and **27b** are almost devoid of activity as inhibitors of any of the cysteine proteases tested (with the exception of **26b** vs papain). On the other hand, the L-hPhe analogs **28b** and **29b** are excellent inhibitors of cruzain, albeit with lower selectivity for cruzain vs cathepsin B or papain than was the case with **12b**.

The L-amino acid epoxysuccinates **12b**, **28b** and **29b** may bind to cruzain the same way that E-64 binds to papain,[50] with the hydroxylated P_3 substituent binding in the S_3 pocket as predicted by our ligand design. The increased potency of **28b** and **29b** for cruzain (compared to **12b**) is fully consistent with this analysis. On the other hand, the poor activity of the D-h-Phe containing analogs **11c**, **26b**, and **27b** led us to suspect that the binding modes (for **11c**) used in our molecular modelling study may be incorrect. Indeed, X-ray crystallographic characterization of the cruzain·**11c** and cruzain·**12b** structures verify distinctly different binding modes for these inhibitors; these structures will be reported in due course.[72]

Table 3. Second order rate constants, k_{inact}/K ($s^{-1}\,M^{-1}$), for inactivation of cruzain, bovine cathepsin B and papain by E-64 analogs **26b**–**29b**

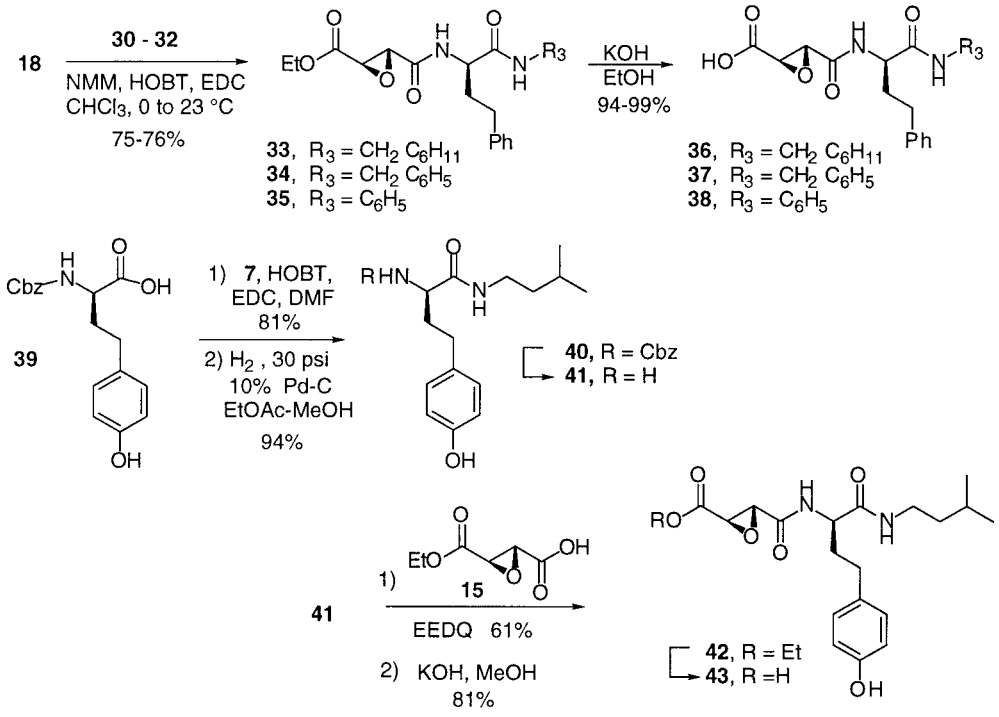
Inhibitor	P_2	P_3	k_{inact}/K_i ($M^{-1}\,s^{-1}$)		
	Amino acid	Residue	vs cruzain	vs cath B	vs papain
26b	D-hPhe	$-(CH_2)_4OH$	2500	1050	25 000
27b	D-hPhe	$-(CH_2)_5OH$	5000	7600	4300
28b	L-hPhe	$-(CH_2)_4OH$	91 500	32 500	39 000
29b	L-hPhe	$-(CH_2)_5OH$	96 200	41 700	54 200

We briefly explored the tolerance of other P_3 hydrophobic groups in additional analogs of **11c**. Thus, coupling of acid **18** with cyclohexylmethylamine (**30**), benzylamine (**31**) and aniline (**32**) followed by hydrolysis afforded the corresponding acids **36**–**38**. In search of ways to improve the water solubility of these compounds we substituted the amino acid D-homotyrosine (D-hTyr) for D-hPhe at the P_2 position. We used Yokoo's method[64] for the synthesis of the D-hTyr derivative (**43**) (Scheme 5).

Preliminary IC_{50} data obtained with these compounds suggested that only **38**, containing a P_3 aniline residue, is a satisfactory inhibitor of cruzain. Reproducible kinetic assays performed on **38** established that the potency (k_{ass}=66,000 $s^{-1}\,M^{-1}$) is truly comparable to that of **5**, and **12b**. In contrast, replacement of the D-hPhe amino acid for D-hTyr was tolerated but with a decrease in activity in the resulting inhibitor **43** (k_{ass}=32,500 $s^{-1}\,M^{-1}$) (Table 4).

Probing the S′1 site of cruzain with *O*-benzyl hydroxamate derivatives

E-64 and other naturally occurring peptidyl epoxysuccinates



Scheme 5.

bind only in the S subsites of the targeted cysteine protease. However, the literature contains a growing number of examples in which increased potency and selectivity of inhibitors has been achieved vs cysteine proteases of the papain family by taking advantage of binding interactions in both the S and S′ subsites. For example, potent peptidomimetic inhibitors that span the active site of cathepsin K have been prepared by the SmithKline group.[73] Similarly, the development of synthetic derivatives that bind in the S′ sites of cathepsin B[42,43] paved the way for the successful design of E-64 analogs targeting the entire active site of cathepsins B and L.[46–48]

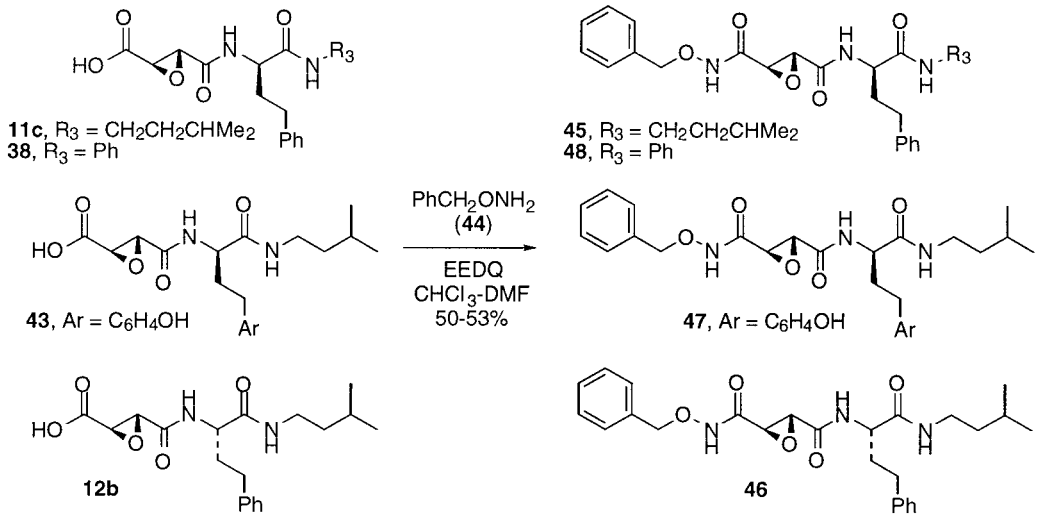
In an effort to explore interactions of synthetic E-64 analogs with the cruzain S′ sites we were attracted by the work of Rich and Meara who replaced the free carboxylate of E-64c (**5**) by other functionalities and found that a hydroxamic acid unit is the closest in potency to the parent acid for inhibition of papain. In contrast a primary amide function lowered the potency by about two orders of magnitude.[74] In analogous way, substrate-like peptidyl *O*-acyl hydroxamates have been used as very potent cysteine protease inhibitors.[75] We thus decided to probe interactions of the shallow, hydrophobic S'_1 site of cruzain with the *O*-benzyl hydroxamates **45**–**48** prepared by treatment of the parent acids with *O*-benzyl hydroxylamine (**44**) and EEDQ[76] (Scheme 6).

The results of kinetic evaluation of **45**–**48** with cruzain are given in Table 5. The D-hPhe hydroxamate inhibitors **45** and **48** display increased activity compared to the parent acids **11c** and **38**. *However, and most surprisingly, the L-hPhe derivative **46** proved to be a very weak inhibitor*. Once again this divergent set of results suggests that the epimeric inhibitors bind to cruzain in different ways. It is also evident from these results that iterative structural modifications in the D-hPhe series can not be applied directly to the L-hPhe series, or vice versa. An indication of the importance of the hydroxamic acid functionality to the potency of the inhibitor is given by comparison of the IC_{50} values of the amide **49** and *O*-benzyl hydroxamate ester **45** isosteres. The amide suffers a most significant three order of magnitude drop in activity (IC_{50} determination).

The D-hTyr derivative **47** is the most potent inhibitor of cruzain to emerge from this series. We studied this inhibitor against several other cysteine proteases: the cathepsin B-like protease from the parasite *Leishmania major*,[29] bovine cathepsin B[61] and papain (Table 6). That **47** is highly selective for cruzain vs bovine cathepsin B, which is highly homologous to cruzain, may have significance for further development of this inhibitor series targeting Chaga's disease. Cathepsin B is an important cysteine protease and alterations of its metabolism have been associated with pathological problems.[61]

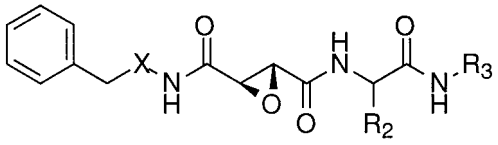
Table 4. IC_{50} (nM) and k_{ass} (M^{-1} sec^{-1}) values for compounds **36**–**38** and **43**. k_{ass} is a second order inhibition rate constant, and is used in cases where saturation kinetics demonstrating reversible inhibitor binding cannot be determined (Ref. [66])

Inhibitor	P_3	P_2	IC_{50} vs cruzain (Nm)	k_{assoc} (M^{-1} s^{-1})
36	$-CH_2C_6H_{11}$	$-CH_2CH_2Ph$ (D-hPhe)	<1000	nd
37	$-CH_2C_6H_5$	$-CH_2CH_2Ph$ (D-hPhe)	~100	nd
38	$-C_6H_5$	$-CH_2CH_2Ph$ (D-hPhe)	<10	66 000
43	$-CH_2CH_2CHMe_2$	$-CH_2CH_2C_6H_4OH$ (D-hTyr)	~50	32 500



Scheme 6.

Table 5. IC_{50} and kinetic data for inhibitors **45**–**49** vs. cruzain. For compound **47** the k_{ass} value is reported; nd=not determined



Inhibitor	X	R_2	P_2 amino acid	R_3 side chain	k_{inact}/K vs cruzain	IC_{50} vs cruzain (nM)
45	O	$-CH_2CH_2Ph$	(D-hPhe)	$-CH_2CH_2CHMe_2$	118 000	6
46	O	$-CH_2CH_2Ph$	(L-hPhe)	$-CH_2CH_2CHMe_2$	6000	nd
47	O	$-CH_2CH_2C_6H_4OH$	(D-hTyr)	$-CH_2CH_2CHMe_2$	441 600	<10
48	O	$-CH_2CH_2Ph$	(D-hPhe)	$-C_6H_5$	179 700	2
49	CH_2	$-CH_2CH_2Ph$	(D-hPhe)	$-CH_2CH_2CHMe_2$	nd	6000

Table 6. Activity of inhibitor **47** against several cysteine proteases

	Cruzain	Cath. B (bovine)	Cath. B (Leish)	Papain
k_{inact}/K_i (s^{-1} M^{-1})	441 600	1785	8594	1448
Cruzain/enzyme relative selectivity	1	247:1	51:1	305:1

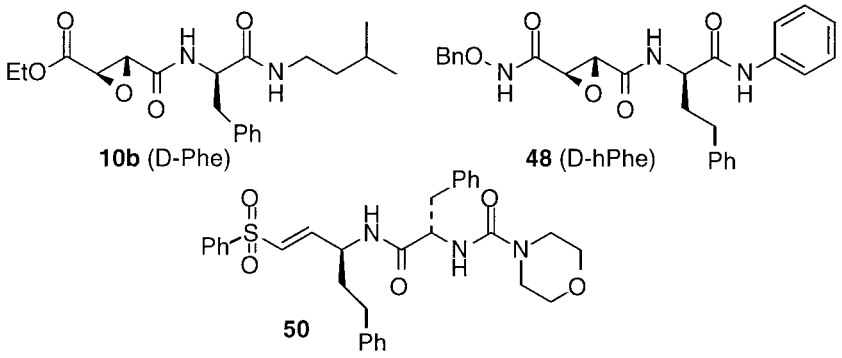
Effect of epoxysuccinyl inhibitors in vivo vs *T. Cruzi*

Ethyl esters **10a**–**e**, **12a**, **26a**, **27a**, **28a**, **29a**, **33**–**35** and hydroxamates **45**–**48** were tested against J744 cells infected with *T. cruzi*. in a standard tissue culture assay.[32] Ethyl esters were used as prodrugs for all epoxysuccinic acid inhibitors, since the carboxylic acids themselves are inactive in vivo (presumably due to problems with transport across the cell membrane). In spite of the promising results for the inhibition of cruzain in vitro, most of the compounds displayed only marginal or no activity against *T. cruzi*in tissue culture. In just two cases (**10b** and **48**) did the inhibitor prolong the lifetime of the infected cells (Table 7). Compound **10b** extended the life of the infected cells for 16 days at a concentration of 40 μM whereas intermediate **48** extended the lifetime for 13 days at 10 μM concentration. In contrast, the reference compound **50**[24,25,77–79] which has proved to cure Chagas' disease in a mouse model,[13] extended the lifetime of infected J744 cells for>28 days at 10 μM inhibitor concentration. Most of the compounds tested suffer from low water solubility that may contribute to their lack of activity in vivo. Despite its high potency against cruzain, inhibitor **47** was inactive against the parasite in vivo. These facts underline the challenge to improve the low bioavailability of these compounds by further structural modifications.

Conclusion

Iterative modifications of the structure of E-64c via molecular modeling and ligand design within the active site of cruzain, combined with the synthesis of new epoxysuccinyl inhibitors designed to explore P_3/S_3 binding interactions have led to potent D-hPhe (**45**, **48**) and D-hTyr (**43**, **47**) containing epoxysuccinate inhibitors of cruzain.

Table 7. Effect of epoxysuccinyl inhibitors on survival of J744 macrophages infected with *T. cruzi* trypomastigotes, treated daily with a solution of inhibitor (typically 10 μM). Survival time is defined as the time before the cell monolayer is destroyed by infection.[15,32] Untreated cells are completely destroyed after 6 days in this assay



Inhibitor	Concentration (μM)	Survival time (days)
10b	40	16
48	10	13
50	10	>28

However, the actual mode of binding of these inhibitors to cruzain differs from the model from which they were derived.[72] Evaluation of these compounds in vivo shows that only **10b** and **48** display activity against *T. cruzi* in tissue culture. Compound **47** is the most potent peptidyl epoxysuccinate inhibitor prepared in this series. The synthesis of a larger number of *O*-alkyl hydroxamic acid derivatives offers the potential for discovery of very potent and specific epoxysuccinyl inhibitors of other important cysteine proteases. Such studies are in progress and will be reported in due course.

Experimental

General

^{1}H NMR spectra were measured at 400 MHz on a Varian VXR-400 instrument or at 500 MHz on a Varian Inova 500 instrument. Residual chloroform (δ 7.27 ppm), methanol (δ 3.31 ppm) or Me_4Si in DMSO-d_6 were used as internal references. ^{13}C NMR spectra were measured at 100 or 125 MHz using residual chloroform (77.0 ppm) or DMSO (39.5 ppm) as internal reference. Unless otherwise stated, the ^{1}H and ^{13}C NMR spectra were measured at 500 and 100 MHz respectively. Infrared spectra were recorded on a Perkin–Elmer Spectrum 1000 spectrophotometer. High resolution mass spectra (HRMS) were measured on a Micromass Corp. VG 70-250-S spectrometer at the University of Michigan Mass Spectrometry Laboratory. Chemical ionization (CI) mass spectra were obtained using NH_3 as the reagent gas. Optical rotations were measured on a Rudolph Autopoll III polarimeter using a quartz cell of 1 mL capacity and a 10 cm path length. Melting points were determined in a Thomas Hoover apparatus and are uncorrected. L- and D-homophenylalanine (hPhe), D-homotyrosine (D-hTyr), 1-hydroxybenzotriazole hydrate (HOBT·H_2O), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC·HCl) and *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) were purchased from Advanced ChemTech (Kentucky, USA). *N*-methylmorpholine (NMM), chloroform (stabilized with amylene) and *N*,*N*′-dimethyl formamide (DMF) were dried and stored over 4 Å molecular sieves. IC_{50} determinations and kinetic analysis with cruzain were performed as previously described.[32] The experimental conditions for evaluation of the effect of the inhibitors described in this paper on J774 macrophages infected with *T. cruzi* trypomastigotes have been described by Engel et al.[13]

Compounds **10a**–**e** were prepared from the *p*-nitrophenol ester **9** and the free amines derived from the peptidomimetics **8a**–**e** by the method reported by Yokoo for the synthesis of **4**.[58] Yields are indicated in parentheses.

(2*S*,3*S*)-3-[[[(1*R*)-3-Methyl-1-[[(3-methylbutyl)amino]-carbonyl]butyl]amino]carbonyl]-oxiranecarboxylic acid ethyl ester (10a). Obtained as a white solid (89%): mp 135°C; $[\alpha]_D$=+82.4° (*c*=2.1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.45 (d, *J*=8.5 Hz, 1H), 5.80 (bs, 1H), 4.35 (m, 1H), 4.27 (m, 2H), 3.68 (d, *J*=2 Hz), 3.52 (d, *J*=2 Hz, 1H), 3.26 (m, 2H), 1.61 (m, 4H), 1.38 (q, *J*=7 Hz, 2H), 1.32 (t, *J*=7 Hz, 3H), 0.93 (m, 12H);^{13}C NMR ($CDCl_3$) δ 170.96, 166.46, 166.00, 62.17, 53.70, 52.63, 51.51, 41.26, 38.29, 37.93, 25.82, 24.77, 22.70, 22.33, 22.22, 13.96; IR ($CHCl_3$) ν cm^{-1} 3450, 3410, 1760, 1685, 1535, 1475, 1380, 1320, 1090, 1035, 915; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{17}H_{31}N_2O_5$: 343.2232, found: 343.2238.

(2*S*,3*S*)-3-[[[(1*R*)-2-[3-Methylbutyl)amino]-2-oxo-1-(phenylmethyl)ethyl]amino]carbonyl-oxiranecarboxylic acid ethyl ester (10b). Obtained as a white solid (95%): mp 124°C; $[\alpha]_D$=+31.0° (*c*=2.0; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 (m, 5H), 6.24 (d, *J*=8 Hz, 1H), 5.26 (bs, 1H), 4.45 (m, 1H), 4.25 (m, 2H), 3.65 (d, *J*=2 Hz, 1H), 3.49 (d, *J*=2 Hz, 1H), 3.11 (m, 3H), 2.95 (dd, *J*=9, 13 Hz, 1H), 1.37 (m, 1H), 1.32 (t, *J*=7 Hz, 3H), 1.15 (q, *J*=7 Hz, 2H), 0.83 (d, *J*=7 Hz, 3H), 0.82 (d, *J*=7 Hz, 3H);^{13}C NMR ($CDCl_3$) δ 169.66, 166.51, 165.74, 136.19, 129.17, 128.65, 127.09, 62.21, 54.49, 53.64, 53.58, 52.61, 52.56, 38.79, 37.89, 37.68, 25.46, 22.24, 13.96; IR ($CHCl_3$) ν cm^{-1} 3420,

3375, 1745, 1670, 1520, 1460, 1360, 1310, 920, 890; HRMS, CI (NH_3) $[M+H]^+$ calcd for C_{20} $H2_9N_2O_5$ 377.2076, found: 377.2077.

(2*S*,3*S*)-3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-3-phenylpropyl]amino]carbonyl]-oxiranecarboxylic acid ethyl ester (10c). Obtained as a white solid (92%): mp 174–176°C; $[\alpha]_D$=+44.8° (*c*=2.3; $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 (m, 5H), 6.61 (d, *J*=8.4 Hz, 1H), 5.75 (bs, 1H), 4.27 (m, 3H), 3.65 (d, *J*=1.6 Hz, 1H), 3.50 (d, *J*=1.6 Hz, 1H), 3.24 (m, 2H), 2.65 (m, 2H), 2.14 (m, 1H), 1.98 (m, 1H), 1.56 (m, 1H), 1.35 (q, *J*=7 Hz, 2H), 1.30 (t, *J*=7 Hz, 3H), 0.90 (d, *J*=7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.23, 166.49, 165.96, 140.37, 128.56, 128.28, 126.29, 62.28, 53.71, 53.61, 52.68, 52.61, 52.46, 38.20, 37.91, 33.93, 31.66, 25.73, 22.32, 13.98; IR ($CHCl_3$) ν cm^{-1} 3430, 3380, 1745, 1675, 1520, 1450, 1360, 1300, 1080, 1020, 890; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{21}H_{31}N_2O_5$: 391.2233, found: 391.2244. Anal. Calcd for $C_{21}H_{30}N_2O_5$: C, 64.59; H, 7.74; N, 7.18. Found: C, 64.75; H, 7.92; N, 7.13.

(2*S*,3*S*)-3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-2-(phenylmethoxy)ethyl]amino]carbonyl]-oxiranecarboxylic acid ethyl ester (10d). Obtained as a white solid (88%): mp 131°C; $[\alpha]_D$=+13.0° (*c*=2.1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.34 (m, 5H), 6.9 0 (d, *J*=7 Hz, 1H), 6.25 (m, 1H), 4.59 (d, *J*=12 Hz, 1H), 4.52 (d, *J*=12 Hz, 1H), 4.45 (ddd, J_a=4.4 Hz, J_b=8.8 Hz, J_c=9.2 Hz, 1H), 4.25 (m, 2H), 3.83 (dd, J_a=4.4 Hz, J_b=9.2 Hz, 1H), 3.67 (d, *J*=1.6 Hz, 1H), 3.67 (d, *J*=1.6 Hz, 1H), 3.49 (app t, *J*=8.8 Hz, 1H), 3.25 (m, 2H), 1.54 (m, 1H), 1.34 (q, *J*=7 Hz, 2H), 1.30 (t, *J*=7 Hz, 3H), 0.87 (d, *J*=6.8 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 168.76, 166.49, 166.00, 136.99, 128.54, 128.10, 127.84, 73.53, 68.99, 62.26, 53.69, 52.71, 51.69, 38.12, 37.93, 25.65, 22.31, 22.29, 13.98; IR ($CHCl_3$) ν cm^{-1} 3360, 1740, 1665, 1510, 1460, 1360, 1300, 1080, 1015, 890; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{21}H_{31}N_2O_6$: 407.2182, found: 407.2192.

(2*S*,3*S*)-3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-2-methyl-2-(phenyl methoxy)ethyl]amino]carbonyl]-oxiranecarboxylic acid ethyl ester (10e). Obtained as a white solid (96%): mp 171°C; $[\alpha]_D$=+23.5° (*c*=2.0; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ7.35 (m, 5H), 7.06 (d, *J*=6.4 Hz, 1H), 6.39 (m, 1H), 4.68 (d, *J*=11.6 Hz, 1H), 4.62 (d, *J*=11.6 Hz, 1H), 4.49 (dd, J_a=3.2 Hz, J_b=6.8 Hz, 1H), 4.26 (M, 2H), 4.05 (m, 1H), 3.68 (d, *J*=1.6 Hz, 1H), 3.58 (d, *J*=1.6 Hz, 1H), 3.29 (m,1H), 3.20 (m,1H), 1.53 (m, 1H), 1.31 (m, 5H), 1.11 (d, *J*=6 Hz,3H), 0.87 (d, *J*=6.4 Hz, 3H), 0.85 (d, *J*=6.4 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 167.80, 166.52, 165.86, 137.55, 128.52, 128.00, 127.78, 73.73, 71.48, 62.21, 54.98, 53.68, 52.70, 38.07, 37.78, 25.58, 22.26, 22.22, 14.61, 13.98; IR ($CHCl_3$) ν cm^{-1} 3380, 1745, 1670, 1515, 1460, 1360, 1300, 1090, 1020, 895; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{22}H_{33}N_2O_6$: 421.2338, found: 421.2319.

Synthesis of compounds 11a–e

Compounds **11a–e** were prepared by saponification of the corresponding ethyl esters **10a–e** with KOH–ethanol as described by Yokoo et al.[58]

(2*S*,3*S*)-3-[[[(1*R*)-3-Methyl-1-[[(3-methylbutyl)amino]-carbonyl]butyl]amino]carbonyl]-oxiranecarboxylic acid (11a). Obtained from **10a** as a white solid (99%): mp 58°C; $[\alpha]_D$=+96.0° (*c*=1.0; ethanol); ^{1}H NMR ($CDCl_3$) δ 7.30 (d, *J*=8.5 Hz, 1H), 6.63 (s, 1H), 4.49 (q, *J*=7 Hz, 1H), 3.70 (d, *J*=1.5 Hz, 1H), 3.60 (d, *J*=1.5 Hz, 1H), 3.30 (m, 1H), 3.20 (m, 1H), 1.60 (m, 4H), 1.40 (m, 2H), 0.92 (m, 12H); ^{13}C NMR ($CDCl_3$) δ 172.16, 169.45, 166.66, 53.49, 52.29, 51.80, 41.00, 37.85, 25.77, 24.70, 22.57, 22.36, 22.28, 22.19; IR (KBr) ν cm^{-1} 3294, 3089, 2956, 1739, 1654, 1560, 1467, 1388, 1369, 1232, 1171, 898, 867, 652; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{15}H_{27}N_2O_5$: 315.1913, found: 315.1918.

(2*S*,3*S*)-3-[[[(1*R*)-2-[3-Methylbutyl)amino]-2-oxo-1-(phenylmethyl)ethyl]amino]carbonyl-oxiranecarboxylic acid (11b). Obtained from **10b** as a white solid (93%): mp 94–95°C; $[\alpha]_D$=+35.8° (*c*=2.4; $CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 13.44 (s, 1H), 8.70 (d, *J*=8 Hz, 1H), 8.03 (t, *J*=5 Hz, 1H), 7.21 (m, 5H), 4.75 (m, 1H), 3.61 (d, *J*=1.5 Hz, 1H), 3.31 (d, *J*=1.5 Hz, 1H), 3.04 (m, 2H), 2.97 (dd, J_a=6 Hz, J_b=14 Hz, 1H), 2.78 (dd, J_a=6 Hz, J_b=14 Hz, 1H), 1.47 (m, 1H), 0.83 (m, 6H); ^{13}C NMR (DMSO-d_6) δ 169.96, 168.74, 164.98, 137.49, 129.12, 128.07, 126.36, 54.25, 52.51, 51.08, 37.89, 36.75, 24.97, 22.36; IR (KBr) ν cm^{-1} 3298, 3089, 2958, 1736, 1648, 1546, 1498, 1458, 1387, 1368, 1228, 897, 860, 745, 700, 650; HRMS CI (NH_3) $[M+H]^+$ calcd for $C_{18}H_{25}N_2O_5$: 349.1757, found: 349.1760.

(2*S*,3*S*)-3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-3-phenylpropyl]amino]carbonyl]-oxiranecarboxylic acid (11c). Obtained from **10c** as a white solid (89%): mp 138–140°C; $[\alpha]_D$=+39.2° (*c*=1.0; $CHCl_3$); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 7.56 (d, *J*=8.5 Hz, 1H), 7.28 (m, 1H), 7.22 (m, 2H), 7.11 (m, 3H), 4.36 (t, *J*=7 Hz, 1H), 3.61 (s, 1H), 3.53 (s, 1H), 3.21 ((m, 1H) 3.13 (m, 1H), 2.58 (t, *J*=7 Hz, 2H), 2.03 (m, 1H), 1.93 (m, 1H), 1.54 (m, 1H), 1.33 (q, *J*=7 Hz, 2H), 0.85 (d, *J*=7 Hz, 6H); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 170.92, 168.84, 166.39, 140.31, 128.30, 128.10, 126.00, 53.23, 52.49, 52.14, 37.80, 37.70, 33.71, 31.54, 25.45, 22.12, 22.06; IR ($CHCl_3$) ν cm^{-1} 3420, 3380, 1735, 1670, 1520, 1450, 1230, 1030, 890; FAB $[M+H]^+$ calcd for $C_{19}H_{27}N_2O_5$: 363.1913, found: 363.1909.

(2*S*,3*S*)-3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-2-(phenylmethoxy)ethyl]amino]carbonyl]-oxiranecarboxylic acid (11d). Obtained from **10d** as an oil (93%): $[\alpha]_D$=+10.8° (*c*=1.2; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 11.00 (bs, 1H), 7.54 (d, *J*=8 Hz, 1H), 7.30 (m, 5H), 6.95 (t, *J*=5 Hz, 1H), 4.66 (dd, J_a=5 Hz, J_b=12 Hz, 1H), 4.53 (d, *J*=12 Hz, 1H), 4.50 (d, *J*=12 Hz, 1H), 3.74 (m, 1H), 3.72 (d, *J*=1.5 Hz, 1H), 3.61 (d, *J*=1.5 Hz, 1H), 3.59 (m, 1H), 3.22 (m, 2H), 1.54 (m, 1H), 1.33 (q, *J*=7 Hz, 2H), 0.86 (d, *J*=6 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 169.61, 169.07, 166.82, 136.93, 128.44, 127.99, 127.75, 73.33, 68.99, 53.42, 52.29, 38.14, 37.77, 25.55, 22.26, 22.22; IR (KBr) ν cm^{-1} 3299, 3090, 2957, 2871, 1739, 1651, 1548, 1455, 1386, 1367, 1210, 1108, 897, 859, 736, 698; HRMS CI (NH_3) $[M+H]^+$ calcd for $C_{19}H_{27}N_2O_6$: 379.1862, found: 379.1874.

(2*S*,3*S*)-3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-2-methyl-2-(phenylmethoxy)ethyl]amino]carbonyl]-oxiranecarboxylic acid (11e). Obtained from **10e** as an oil (98%):

$[\alpha]_D$=+15.0° (*c*=1.0; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 10.06 (bs, 1H), 7.45 (d, *J*=8 Hz, 1H), 7.33 (m, 5H), 6.74 (t, *J*=5.5 Hz, 1H), 4.60 (m, 3H), 4.08 (m, 1H), 3.74 (*J*=1.5 Hz, 1H), 3.62 (d, *J*=1.5 Hz, 1H), 1.53 (m, 1H), 1.32 (m, 2H), 1.13 (d, *J*=6 Hz, 3H), 0.86 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 169.02, 168.64, 166.74, 137.39, 128.52, 128.02, 127.85, 73.89, 71.47, 55.63, 53.49, 52.37, 25.57, 22.25, 22.21, 15.03; IR (KBr) ν cm^{-1} 3370, 2960, 1735, 1650, 1515, 1450, 1380, 1230, 1075, 1030, 890, 690; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{20}H_{29}N_2O_6$: 393.2018, found: 393.2012.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[(phenylmethoxy)carbonyl]-propyl]amino]carbonyl]-oxiranecarboxylic acid ethyl ester (16). To a solution of the *p*-toluensulfonic acid salt of D-homophenylalanine benzyl ester **13**[68] (2.14 g, 4.8 mmol), monoethyl epoxysuccinate **15**[58] (0.78 g, 4.8 mmol), HOBT·H_2O (0.74 g, 4.8 mmol) and *N*-methylmorpholine (0.54 mL, 4.8 mmol), in dry chloroform (10.0 mL) under nitrogen at 0°C was added EDC·HCl (1.02 g, 5.2 mmol). The mixture was stirred at 0°C for 1 h and then 7 h at 23 0°C. The mixture was then partitioned between EtOAc and water. The organic portion was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (eluted with 7:3 hexanes–EtOAc) 7:3 to give 658 mg (80%) of **16** as a white solid: mp 76–78°C; $[\alpha]_D$=+47.7° (*c*=3.0; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.41–7.10 (m, 10H), 6.45 (d, *J*=9 Hz, 1H), 5.18 (d, *J*=12.5 Hz, 1H), 5.12 (d, *J*=12.5 Hz, 1H), 4.68 (m, 1H), 4.27 (m, 2H), 3.69 (d, *J*=2 Hz, 1H), 3.50 (d, *J*=2 Hz, 1H), 2.61 (m, 2H), 2.22 (m, 1H), 2.03 (m, 1H), 1.33 (t, *J*=7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.22, 166.38, 165.86, 140.09, 134.97, 128.71, 128.67, 128.55, 128.43, 128.33, 126.37, 67.46, 62.13, 53.71, 52.73, 51.54, 33.47, 31.52, 14.03; IR ($CHCl_3$) ν cm^{-1} 3684, 3402, 3025, 3016, 1745, 1690, 1525, 1227, 1205, 1028, 929, 796, 719; HRMS, CI (NH_3) $[M]^+$ calcd for $C_{23}H_{25}NO_5$ 411.1675, found: 411.1670. Anal. Calcd for $C_{23}H_{25}NO_6$: C, 67.14; H, 6.12; N, 3.40. Found: C, 67.13; h 6,.38; N, 3.46.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-(carboxyl)propyl]amino]-carbonyl]-oxiranecarboxylic acid ethyl ester (18). A mixture of benzyl ester **16** (0.60 g, 1.4 mmol) and 10% Pd/C (200 mg) in 50 mL of EtOAc and 50 mL of methanol was shaken on a Parr apparatus under hydrogen (40 psi) for 15 min. The mixture was purged with nitrogen, the catalyst removed by filtration through a pad of Celite and the solution concentrated in vacuo to afford 0.45 g (97%) of **18** as a white solid: mp 124–126°C; $[\alpha]_D$=+58.7° (*c*=3.1; CH_3OH); ^{1}H NMR ($CDCl_3$) δ 10.6 (bs, 1H), 7.31–7.17 (m, 5H), 6.64 (d, *J*=8 Hz, 1H), 4.64 (m, 1H), 4.27 (m, 2H), 3.73 (d, *J*=2 Hz, 1H), 3.56 (d, *J*=2 Hz, 1H), 2.71 (t, *J*=7 Hz, 2H), 2.27 (m, 1H), 2.07 (m, 1H), 1.31 (t, *J*=7 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 175.65, 166.45, 166.42, 139.91, 128.59, 128.33, 126.44, 62.42, 53.61, 52.77, 51.42, 33.14, 31.61, 13.98; IR (KBr) ν cm^{-1} 3382, 3351, 2929, 1750, 1636, 1545, 1498, 1455, 1323, 1235, 1211, 1128, 1102, 1024, 904; HRMS, EI, M^+ calcd for $C_{16}H_{19}NO_6$ 321.1212, found: 321.1200.

4-(*t*-Butyl-dimethyl-silanyloxy)-butylamine (20). To a 0°C solution of 4-amino-1-butanol (5.0 g, 56.1 mmol), triethylamine (6.2 g, 61.7 mmol), and 4-dimethylaminopyridine (34 mg, 0.3 mmol) in 50 mL of CH_2Cl_2 under nitrogen was added *tert*-butyldimethylchloro silane (8.88 g, 58.9 mmol). The mixture was stirred for 0.5 h, then was allowed to warm to 23°C and stirred for 3.5 h. After this time the reaction mixture was poured into cold water and extracted with CH_2Cl_2. The organic portion was washed with $NaHCO_3$, brine and dried over Na_2SO_4. The solvent was evaporated in vacuo and the residue was distilled bulb to bulb (150°C, 0.01 mm Hg) to obtain 9.7 g (85%) of **20** as a slightly yellow semi-solid: ^{1}H NMR ($CDCl_3$) δ 3.52 (t, *J*=6.5 Hz, 2H), 2.71 (bs, 2H), 2.61 (t, *J*=6.5 Hz, 2H), 1.43 (m, 4H), 0.80 (s, 9H), −0.05 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 62.78, 41.59, 29.99, 29.54, 25.76, 25.65, 18.11, −5.50; IR (neat) ν cm^{-1} 3315, 2953, 2930, 2858, 1647, 1577, 1486, 1471, 1383. 1361, 1307, 1255, 1191, 1102, 991, 938, 836, 774; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{10}H_{26}NOSi$ 204.1783, found: 204.1778.

5-(*t*-Butyldimethylsilanyloxy)-pentylamine (21). Obtained as an oil in 86% yield from 5-amino-1-pentanol by the method described above: ^{1}H NMR ($CDCl_3$) δ 3.49 (t, *J*=6.5 Hz, 2H), 2.95 (bs, 2H), 2.58 (t, *J*=6.5 Hz, 2H), 1.42 (m, 4H), 1.25 (m, 2H), 0.79 (s, 9H), −0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 62.80, 41.53, 32.63, 32.38, 25.73, 25.61, 22.87, 18.09, −5.52; IR (neat) ν cm^{-1} 3306, 2930, 2858, 1578, 1472, 1387, 1361, 1309, 1255, 1187, 1102, 1006, 938, 835, 775; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{11}H_{28}NOSi$ 218.1940, found: 2218.1932.

(2*S*,3*S*)-3-[[[(1*R*)3-Phenyl-1-[[(4-*t*-butyldimethylsilanoxy-butyl)amino]carbonyl]propyl)amino]carbonyl]-oxirane-carboxylic acid ethyl ester (22). To a solution of the amine **20** (0.20 g, 1 mmol), acid 18 (0.32 g, 1 mmol), HOBT·H_2O (0.15 g, 1 mmol), and *N*-methylmorpholine (0.1 mL) in $CHCl_3$ (5 mL) under nitrogen at 0°C was added EDC (0.21 g, 1.1 mmol). The resulting mixture was stirred 1 h at this temperature then at 23°C for 9 h. The mixture was then partioned between EtOAc and water. The organic portion was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (elution with hexanes–EtOAc 7:3) to afford 0.32 g (64%) of 22 as a white solid: mp 114–116°C; $[\alpha]_D$=+27.1° (*c*=2.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.31–7.16 (m, 5H), 6.60 (d, *J*=8 Hz, 1H), 5.87 (t, *J*=5.5 Hz, 1H), 4.29 (m, 3H), 3.67 (d, *J*=2 Hz, 1H), 3.64 (t, *J*=6 Hz, 2H), 3.52 (d, *J*=2 Hz, 1H), 3.31 (m, 1H), 3.26 (m, 1H), 2.67 (m, 2H), 2.16 (m, 1H), 2.00 (m, 1H), 1.56 (m, 4H), 1.32 (t, *J*=7 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 6H); ^{13}C NMR ($CDCl_3$) (75 MHz) δ 170.25, 166.50, 165.88, 140.38, 128.54, 128.27, 126.25, 62.54, 62.23, 53.67, 52.63, 52.52, 39.40, 34.08, 29.97, 26.03, 25.90, 18.27, 13.99, −5.35; IR ($CHCl_3$) ν cm^{-1} 3400, 3020, 2940, 2870, 1755, 1675, 1525, 1475, 1460, 1395, 1360, 1260, 1100, 1030, 900, 845, 705; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{26}H_{43}N_2O_6$ Si 507.2890, found: 507.2908.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(5-*t*-butyldimethylsilanoxy-pentyl)amino]carbonyl]propyl)amino]carbonyl]-oxirane-carboxylic acid ethyl ester (23). Obtained in 66% yield from acid **18** and amine **21** by the method described above: mp 125–126°C; $[\alpha]_D$=+27.7° (*c*=2.6; $CHCl_3$); ^{1}H NMR ($CHCl_3$) δ 7.32–7.17 (m, 5H), 6.58 (d, *J*=8 Hz, 1H),

5.71 (t, J=6 Hz, 1H), 4.28 (m, 3H), 3.67 (d, J=2 Hz, 1H), 3.61 (t, J=6.5 Hz, 2H), 3.51 (d, J=2 Hz, 1H), 3.24 (m, 2H), 2.67 (m, 2H), 2.16 (m, 1H), 2.00 (m, 1H), 1.52 (m, 4H), 1.35 (m, 2H), 1.32 (t, J=7 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.31, 466.51, 165.94, 140.37, 128.53, 128.26, 62.85, 62.23, 53.62, 52.62, 52.57, 52.50, 39.63, 34.01, 32.29, 31.66, 29.17, 25.90, 23.18, 18.28, 13.97, −5.34; IR ($CHCl_3$) ν cm^{-1} 3460, 3400, 1755, 1680, 1530, 1460, 1380, 1310, 1260, 1095, 1035, 905, 845; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{27}H_{45}N_2O_6Si$ 521.3046, found: 521.3047.

(2*S*,3*S*)-3-[[[(1*S*)-3-Phenyl-1-[[(4-*t*-butyldimethylsilanoxybutyl)amino]carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid ethyl ester (24). Obtained in 66% yield from acid **19**[55] and amine **20** by the method described above: mp 120°C; $[\alpha]_D$=+26.5° (c=2.0; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.29–7.13 (m, 5H), 7.00 (d, J=8 Hz, 1H), 6.37 (t, J=5 Hz, 1H), 4.40 (dd, J_a=8 Hz, J_b=10 Hz, 1H), 4.26 (m,2H), 3.66 (d, J=1.5 Hz, 1H), 3.63 (t, J=6 Hz, 2H), 3.33 (m, including d, J=2 Hz, 2H), 3.24 (m, 1H), 2.66 (m, 2H), 2.15 (m, 1H), 2.00 (m, 1H), 1.56 (m, 4H), 1.31 (t, J=7 Hz, 3H), 0.89 (s, 9H), 0.05 (s, 6H); ^{13}C NMR ($CDCl_3$) δ 170.22, 166.54, 165.89, 140.46, 128.55, 128.22, 126.26, 62.55, 62.23, 53.67, 52.67, 52.57, 39.41, 34.03, 31.66, 29.96, 26.00, 25.89, 18.26, 13.99, −5.36; IR ($CHCl_3$) ν cm^{-1} 3328, 3277, 3086, 2930, 2859, 1754, 1690, 1643, 1552, 1497, 1455, 1386, 1338, 1255, 1222, 1195, 1100, 1027, 987, 899, 838, 778, 754, 700; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{26}H_{43}N_2O_6Si$ 507.2890, found: 507.2892.

The preparation of compound **25** has been reported.[55]

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(4-hydroxybutyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid ethyl ester (26a). A 48% solution of HF in water (0.31 mL, 0.84 mmol) was added to a suspension of **22** (0.29 g, 0.56 mmol), in CH_3CN (5 mL) and CH_2Cl_2 (5 mL) at 0°C. The mixture was allowed to warm to 23°C and stirred for 15 min. The mixture was diluted with CH_2Cl_2 (30 mL) and washed twice with concentrated $NaHCO_3$ (20 mL), brine (20 mL), dried over Na_2SO_4 and concentrated in vacuo to afford 0.21 g (96%) of crude **26a** as a white solid that required no further purification: mp 115–116°C; $[\alpha]_D$=+44.4° (c=2.7; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.23 (m, 5H), 6.61 (d, J=9 Hz, 1H), 6.24 (bs, 1H), 4.28 (m, 3H) 3.67 (d, J=2 Hz, 1H), 3.64 (t, J=7 Hz, 2H), 3.54 (d, J=2 Hz, 1H), 3.28 (m, 2H), 2,66 (m, 2H), 2.15 (m, 1H), 1,99 (m, 1H), 1.75 (bs, 1H), 1.59 (m, 4H), 1.31 (t, J=7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.51, 166.57, 166.08, 140.40, 128.56, 128.32, 126.28, 62.31, 62.21, 53.66, 52.63, 33.88, 31.71, 29.70, 26.03, 14.00; IR ($CHCl_3$) ν cm^{-1} 3400, 3310, 1750, 1675, 1530, 1465, 1375, 1315, 1080, 900, 720, 700; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{20}H_{29}N_2O_6$ 393.2025, found: 393.2029.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(5-hydroxypentyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid ethyl ester (27a). Obtained in 98% yield from **23** by the procedure described above: mp 135–136°C; $[\alpha]_D$=+37.4° (c=2.7; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.32–7.17 (m, 5H), 6.61 (d, J=8 Hz, 1H), 5.95 (t, J=5 Hz, 1H), 4.30 (m, 1H), 4.27 (m, 2H), 3.67 (d, J=2 Hz, 1H), 3.65 (t, J=7 Hz, 2H), 3.55 (d, J=2 Hz, 1H), 3.27 (m, 2H), 2.67 (m, 2H), 2.16 (m, 1H), 2.00 (m, 1H), 1.61–1.51 (m, 6H), 1.40 (m, 2H), 1.32 (t, J=7 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.44, 166.55, 166.11, 140.33, 128.61, 128.35, 62.34, 53.70, 52.68, 52.47, 39.41, 33.72, 31.95, 31.68, 28.98, 22.92, 14.00; IR (KBr) ν cm^{-1} 3400, 3020, 1715, 1675, 1535, 1460, 1375, 1090, 1035, 940, 905; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{21}H_{31}N_2O_6$ 407.2182, found: 407.2180.

(2*S*,3*S*)-3-[[[(1*S*)-3-Phenyl-1-[[(4-hydroxybutyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid ethyl ester (28a). Obtained in 98% yield from **24** by the procedure described above: mp 140–142°C; $[\alpha]_D$=+31.9° (c=3.1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.31–7.16 (m, 5H), 6.70 (d, J=8 Hz, 1H), 6.39 (t, J=5 Hz, 1H), 3.67 (t, J=6 Hz, 2H), 3.64 (d, J=1.5 Hz, 1H), 3.30 (m, including d, J=1.5 Hz, 3H), 4.37 (m, 1H), 4.27 (m, 2H), 2.64 (m, 2H), 2.17 (m, 1H), 1.99 (m, 1H), 1.76 (bs, 1H), 1.62 (m, 4H), 1.32 (t, J=6 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.39, 166.55, 166.08, 140.49, 128.63, 128.30, 129.35, 62.35, 62.27, 53.69, 52.66, 39.42, 33.82, 31.75, 29.66, 26.06, 14.03; IR ($CHCl_3$) ν cm^{-1} 3380, 3020, 2940, 1750, 1675, 1535, 1455, 1375, 1310, 1220, 1090, 900, 720, 705; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{20}H_{29}N_2O_6$ 393.2018, found: 393.2012. Anal. Calcd for $C_{20}H_{28}N_2O_6$: C, 61.21; H, 7.19; N, 7.14. Found: C, 61.23; H, 7.19; N, 7.13.

The preparation of compound **29a** has been reported elsewhere.[55]

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(4-hydroxybutyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid (26b). A 1N solution of KOH in EtOH (0.5 mL, 0.5 mmol) was added to a solution of the ethyl ester **26a** (0.20 g, 0.5 mmol) in a mixture of THF (5 mL) and ethanol (5 mL) at 0°C and stirred for 0.5 h. The solvent was evaporated in vacuo and the residue was dissolved in cold water (5 mL) and acidified to pH 1–2 with 10% $KHSO_4$ and extracted with EtOAc (5×10 mL). The combined organic portions were washed with brine, dried over Na_2SO_4 and concentrated in vacuo to obtain 0.19 g (96%) of **26b** as a white solid. mp 88°C; $[\alpha]_D$=+40.7° (c=2.7; CH_3OH); ^{1}H NMR($CDCl_3$) δ 7.76 (d, J=7 Hz, 1H), 7.31 (s, 1H), 7.24 (m, 2H), 7.14 (m, 3H), 4.31 (m, 1H), 3.60 (d, J=1 Hz, 1H), 3.57 (d, J=1 Hz, 1H), 3.54 (t, J=6 Hz, 2H), 3.17 (t, J=7 Hz, 2H), 2.61 (m, 2H), 2.07 (m, 1H), 1.94 (m, 1H), 1.52 (m, 4H); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 171.88, 169.37, 167.13, 140.97, 128.81, 128.63, 126.51, 61.83, 53.17, 53.29, 52.58, 39.60, 34.23, 32.17, 29.91, 25.91; IR (KBr) ν cm^{-1} 3284, 3090, 2928, 1736, 1645, 1560, 1497, 1453, 1351, 1232, 1061, 900, 749, 699; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{18}H_{25}N_2O_6$: 365.1706; found: 365.1695.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(5-hydroxypentyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid (27b). Obtained as an oil in 96% yield from **27a** by the procedure described above: $[\alpha]_D$=+64.0° (c=2.0; CH_3OH); ^{1}H NMR ($CDCl_3$) δ 7.95 (d, J=8.5 Hz, 1H), 7.82 (t, J=5.5 Hz, 1H), 7.24 (m, 2H), 7.15 (m, 3H), 4.33 (dd, J_a=6 Hz, J_b=8 Hz, 1H), 3.61 (d, J=2 Hz, 1H), 3.57 (d, J=2 Hz, 1H), 3.53 (t, J=6.5 Hz, 2H), 3.17 (m, 2H), 2.07 (m,

1H), 1.95 (m, 1H), 1.51 (m, 4H), 1.35(m, 2H); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 172.29, 169.65, 167.41, 128.96, 128.80, 126.65, 62.26, 53.84, 53.64, 52.68, 40.04, 39.92, 34.43, 32.42, 29.36, 23.55; IR (neat) ν cm^{-1} 3276, 3090, 2929, 1734, 1664, 1640, 1556, 1452, 1228, 896, 747, 698; CI (NH_3) $[M+H]^+$ calcd for $C_{19}H_{27}N_2O_6$: 379.1869; found: 379.1871.

(2*S*,3*S*)-3-[[[(1*S*)-3-Phenyl-1-[[(4-hydroxybutyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid (28b). Obtained in 95% yield from **28a** by the procedure described above: mp 82°C; $[\alpha]_D$=+56.0° (*c*=2.5; CH_3OH); ^{1}H NMR ($CDCl_3$) δ 7.92 (d, *J*=8 Hz, 1H), 7.82 (t, *J*=5.5 Hz, 1H), 7.23 (m, 2H), 7.13 (m, 3H), 4.36 (dd, J_a= 6 Hz, J_b=8 Hz, 1H), 3.61 (d, *J*=1.5 Hz, 1H), 3.54 (t, *J*= 6 Hz, 2H), 3.40 (d, *J*=1.5 Hz, 1H), 3.18 (m, 2H), 2.59 (m, 2H), 2.06 (m, 1H), 1.94 (m, 1H), 1.52 (m, 2H); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 172.00, 169.44, 167.24, 141.17, 128.91, 128.71, 126.61, 61.93, 53.85, 53.47, 52.75, 39.73, 34.40, 32.30, 30.04, 26.03; IR (KBr) ν cm^{-1} 3457, 3293, 3088, 2938, 1727, 1646, 1551, 1498, 1453, 1389, 1341, 1266, 1061, 897, 742, 699; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{18}H_{25}N_2O_6$: 365.1706; found: 365.1702.

The preparation of compound **29b** has been reported elsewhere.[55]

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(cyclohexylmethyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid ethyl ester (33). Obtained in 76% yield from acid **18** and cyclohexylmethylamine (**30**) by the procedure described above for the synthesis of **22**. mp 149–151°C; $[\alpha]_D$=+72.5° (*c*=2.4; CH_3OH); ^{1}H NMR ($CDCl_3$) δ 7.31–7.16 (m, 5H), 6.65 (d, *J*=8.5 Hz, 1H), 5.90 (t, *J*=5 Hz, 1H), 4.36 (q, *J*=7 Hz, 1H), 4.27 (m, 2H), 3.67 (d, *J*=2 Hz, 1H), 3.51 (d, *J*=2 Hz, 1H), 3.08 (m, 2H), 2.67 (m, 2H), 2.17 (m, 1H), 1.99 (m, 1H), 1.69 (m, 4H), 1.44 (m, 1H), 1.30 (t, *J*=7 Hz, 3H), 1.21 (m, 4H), 0.90 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 170.39, 166.51, 165.96, 140.42, 128.53, 128.26, 128.24, 62.26, 53.63, 52.58, 52.51, 45.76, 37.75, 33.99, 31.66, 30.73, 30.70, 26.24, 25.68, 13.97; IR (KBr) ν cm^{-1} 3289, 3089, 2924, 2854, 1749, 1665, 1560, 1498, 1446, 1370, 1266, 1224, 1200, 1154, 1025, 974, 898, 842, 697; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{23}H_{33}N_2O_5$ 417.2389, found: 417.2379.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(phenylmethyl)amino]carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid ethyl ester (34). Obtained in 75% yield from acid **18** and benzylamine (**31**) by the procedure described above for the synthesis of **22**: mp 166–168°C; $[\alpha]_D$=+57.3° (*c*=2.2; CH_3OH); ^{1}H NMR ($CDCl_3$) δ 7.37–7.12 (m, 10), 6.63 (d *J*=8.5 Hz, 1H), 6.18 (t *J*=5 Hz, 1H), 4.39 (m, 3H), 4.27 (m, 2H), 3.66 (d, *J*=2 Hz, 1H), 3.48 (d, *J*=2 Hz, 1H), 2.66 (m, 2H), 2.19 (m, 1H), 2.01 (m, 1), 1.33 (t, *J*=7 Hz, 3H); ^{13}C NMR, 125 MHz ($CDCl_3$) δ 170.34, 166.51, 166.03, 140.29, 137.60, 128.70, 128.53, 128.25, 127.66, 127.66, 127.61, 126.25, 62.26, 53.56, 52.54, 52.45, 43.55, 33.90, 31.62, 13.98; IR (KBr) ν cm^{-1} 3286, 3088, 3032, 2926, 1746, 1641, 1551, 1498, 1454, 1384, 1370, 1350, 1222, 1201, 1100, 1029, 981, 897, 744, 697; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{23}H_{27}N_2O_5$ 411.1920, found: 411.1923.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[phenylamino]carbonyl]-propyl)amino]carbonyl]-oxiranecarboxylic acid ethyl ester (35). Obtained in 75% yield from acid **18** and aniline (**32**) by the procedure described above for the synthesis of **22**: mp 173°C; $[\alpha]_D$=+70.0° (*c*=2.0;$CHCl_3$); ^{1}H NMR (DMSO-d_6) δ 10.15 (s, 1H), 8.93 (d, *J*=7.5 Hz, 2H), 7.30 (m, 4H), 7.29 (m, 3H), 7.07 (t, *J*=7 Hz, 1H), 4.48 (m, 1H), 4.20 (m, 2H), 3.80 (d, *J*=1.5 Hz, 1H), 3.66 (d, *J*=1.5 Hz, 1H), 2.70 (m, 1H), 2.61 (m, 1H), 2.05 (m, 1H), 1.97 (m, 1H), 1.25 (m, 3H); ^{13}C NMR (DMSO-d_6) δ 169.75, 167.20, 165.13, 140.96, 138.69, 128.69, 128.33, 128.20, 125.91, 123.51, 119.48, 61.50, 53.59, 52.92, 51.18, 33.56, 31.54, 13.88; IR (KBr) ν cm^{-1} 3271, 3073, 2925, 1733, 1656, 1597, 1542, 1499, 1447, 1372, 1293, 1246, 1216, 1162, 1093, 1031, 971, 894, 738, 691; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{22}H_{25}N_2O_5$ 397.1763, found: 397.1770.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(cyclohexylmethyl)amino]-carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid (36). Obtained in 98% yield by hydrolysis of **33** by the procedure described above for **26b**: mp 88°C (dec).; $[\alpha]_D$=+60.4° (*c*=2.2; CH_3OH); ^{1}H NMR (DMSO-d_6) δ 13.49 (s, 1H), 8.72 (d, *J*=8 Hz, 1H), 8.03 (d, *J*=5.5 Hz, 1H), 7.26 (m, 2H), 7.19 (m,3H), 4.27 (dd, J_a=7.5 Hz, J_b=13 Hz, 1H), 4.02 (q, *J*=7 Hz, 1H), 3.72 (s, 1H), 3.51 (s, 1H), 2.94 (m, 1H), 2.89 (m, 1H), 2.54 (m, 3H), 1.95 m, 1H), 1.85 (m, 1H), 1.65 (m, 4H), 1.39 (m, 1H), 1.16 (m, 4H), 0.85 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 170.52, 168.73, 165.15, 141.14, 128.28, 128.13, 125.81, 52.69, 51.17, 44.73, 37.33, 33.85, 31.41, 30.29, 25.97, 25.33; IR (KBr) ν cm^{-1} 3290, 3088, 2925, 2853, 1736, 1646, 1560, 1498, 1449, 1389, 1225, 1154, 1105, 1030, 980, 896, 747, 698; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{21}H_{29}N_2O_5$ 389.2076, found: 389.2060.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-[[(phenylmethyl)amino]carbonyl]propyl)amino]carbonyl]-oxiranecarboxylic acid (37). Obtained in 94% yield by hydrolysis of **34** by the procedure described above for **26b**: mp 110°C (dec); $[\alpha]_D$=+79.0° (*c*=1.0; CH_3OH); ^{1}H NMR (DMSO-d_6) δ 13.48 (bs, 1H), 8.80 (d, *J*=8 Hz, 1H), 8.60 (s, 1H), 7.25 (m, 10H), 4.03 (m, 1H), 3.73 (s, 1H), 3.54 (s, 1H), 2.61 (m,1H), 2.55 (m, 1H), 2.00 (m, 1H), 1.90 (m, 1H), 1.17 (app. t, *J*=7 Hz, 2H); ^{13}C NMR (DMSO-d_6) δ 170.80, 168.83, 165.40, 141.10, 139.33, 128.31, 128.22, 127.07, 126.73, 125.87, 52.77, 51.22, 42.06, 33.62, 31.45; IR (KBr) ν cm^{-1} 3283, 3066, 2926, 2857, 1736, 1654, 1638, 1542, 1498, 1458, 1225, 1081, 1030, 898, 746, 698; HRMS, CI (NH_3) $[M+H]^+$ calcd for$C_{21}H_{23}N_2O_5$ 383.1607, found: 383.1588.

(2*S*,3*S*)-3-[[[(1*R*)-3-Phenyl-1-(phenylamino)carbonyl]-propylamino]carbonyl]-oxiranecarboxylic acid (38). Obtained in 99% yield by hydrolysis of **35** by the procedure described above for **26b**: mp>200°C (dec); $[\alpha]_D$=+68.0° (*c*=2.0; CH_3OH); ^{1}H NMR (DMSO-d_6) δ 13.50 (s, 1H), 10.14 (s, 1H), 8.90 (d, *J*=8 Hz, 1H), 7.59 (d, *J*=7.5 Hz, 2H), 7.27 (m, 4H), 7.19 (m, 3H), 7.06 (t, *J*=7.5 Hz, 1H), 4.46 (m, 1H), 3.73 (s, 1H), 3.52 (s, 1H), 2.68 (m, 1H), 2.60 (m, 1H), 2.04 (m, 1H), 1.96 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 169.79, 168.73, 165.46, 140.97, 138.69, 128.69, 128.34, 128.21, 125.92, 123.51, 119.52, 53.56, 52.67, 33.57, 31.53; IR (KBr) ν cm^{-1} 3294, 3255, 3063, 2931, 1759, 1655, 1619,

1543, 1498, 1447, 1394, 1313, 1255, 1195, 1094, 1028, 989, 950, 894, 754, 694, 646; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{20}H_{21}N_2O_5$ 369.1450, found: 369.1455.

(*R*)-2-[(Phenylmethyl)carbonyl]amino-4-(4-hydroxyphenyl)-butyric acid (39). To a solution of D-homotyrosine hydrobromide (1.00 g, 3.62 mmol) in H_2O (10 mL) at 0°C benzyl chloroformate (0.67 mL, 4.71 mmol) and 2N NaOH (16 mL, 32 mmol) were added simultaneously under vigorous stirring. The mixture was stirred at this temperature for 2 h then at 23°C for 10 h. The reaction mixture was washed with CH_2Cl_2 then cooled to 0°C, acidified to pH 2 with 1 M $KHSO_4$ and extracted with CH_2Cl_2 (3×50 mL). The combined organic portions were washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (elution with CH_2Cl_2-isopropanol 97:3 containing 0.1% AcOH) to afford 0.49 g (41%) of Cbz-*N*-D-homotyrosine **39** as a syrup: $[\alpha]_D=+8.5°$ (c=2.0; CH_3OH); ^1H NMR (DMSO-d_6) δ 12.48 (bs, 1H), 9.13 (s, 1H), 7.66 (d, J=8 Hz, 1H), 7.33 (m, 5H), 6.95 (d, J=8.5 Hz, 2H), 6.65 (d, J=8.5 Hz, 2H), 5.04 (s, 2H), 3.87 (m, 1H), 2.54 (m, 2H), 1.88 (m, 1H), 1.80 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 173.92, 156.16, 155.43, 137.05, 130.98, 129.16, 128.30, 127.75, 127.66, 115.07, 65.35, 53.20, 32.94, 30.64; IR (neat) ν cm^{-1} 3326, 3033, 2928, 1698, 1614, 1597, 1515, 1455, 1416, 1345, 1228, 1052, 911, 829, 741, 697; HRMS, CI (NH_3) $[M+NH_4]^+$ calcd for $C_{18}H_{23}N_2O_5$ 347.1608, found: 347.1623.

(*R*)-*N*-(3-Methylbutyl)-2-[[(phenylmethyl)carbonyl]amino]-4-(4-hydroxy phenyl)-butyric amide (40). To a solution of **39** (0.44 g, 1.33 mmol), isopentylamine (0.15 mL, 1.33 mmol) and HOBT (0.20 g, 1.33 mmol) in DMF (5 mL) under nitrogen at 0°C was added EDC (0.28 g, 1.47 mmol). The resulting mixture was stirred for 1 h at this temperature, then at 23°C for 10 h. The mixture was then partitioned between H_2O and a mixture of EtOAc–hexanes 4:1. The organic portion was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (elution with a gradient of hexanes–EtOAc 7:3 to 6:4) to afford 0.43 g (81%) of **40** as a white solid: mp 126–128°C; $[\alpha]_D=+9.6°$ (c=2.4; $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.33 (m, 5H), 6.98 (d, J=8 Hz, 2H), 6.74 (d, J=8 Hz, 2H), 5.95 (s, 1H), 5.34 (d, J=7.5 Hz, 1H), 5.12 (dd, $J_{1a}=J_b$=12.5 Hz, 2H), 4.07 (q, J=7.5 Hz, 1H), 3.26 (s, 2H), 2.59 (t, J=7.5 Hz, 2H), 2.12 (m, 1H), 1.92 (m, 1H), 1.59 (m, 1H), 1.37 (m, 2H), 0.91 (d, J=7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 171.61, 156.32, 154.48, 132.15, 129.35, 128.55, 128.26, 128.03, 115.48, 67.23, 54.61, 38.24, 38.01, 34.24, 30.79, 25.82, 22.38; IR ($CHCl_3$) ν cm^{-1} 3310, 2956, 1700, 1653, 1540, 1515, 1456, 1240, 1046, HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{23}H_{30}N_2O_4$ 399.2283, found: 399.2273. Anal. Calcd for $C_{22}H_{30}N_2O_4$: C, 69.32; H, 7.59; N, 7.03. Found: C, 69.45; H, 7.85; N, 6.98.

(*R*)-*N*-(3-Methyl)butyl)-2-amino-4-(4-hydroxyphenyl)-butyric amide (41). A mixture of the benzyl carbamate **40** (0.43 g, 1.08 mmol) and 10% Pd/C (100 mg) in 100 mL of methanol was shaken on a Parr apparatus under hydrogen (40 psi) for 30 min. The mixture was purged with nitrogen, the catalyst was removed by filtration through a pad of celite and the solution concentrated in vacuo to afford 0.28 g (97%) of crude **41** as a white solid which was used immediately in the next reaction: mp 125°C; $[\alpha]_D=+2.2°$ (c=1.8; CH_3OH); ^1H NMR (DMSO-d_6) δ 9.1 (bs, 1H), 7.84 (t, J=5.5 Hz, 1H), 6.95 (d, J=8.5 Hz, 2H), 6.66 (d, J=6.6 Hz, 2H), 3.11 (m, 3H), 2.46 (m, 2H), 1.78 (m, 1H), 1.57 (m, 1H), 1.30 (q, J=7 Hz, 2H), 0.86 (d, J=7 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 174.30, 155.25, 131.87, 128.96, 115.01, 54.24, 38.14, 37.17, 36.48, 30.53, 25.09, 22.32; IR (KBr) ν cm^{-1} 3291, 3097, 2955, 2928, 2872, 1647, 1610, 1560, 1515, 1468, 1388, 1367, 1253, 1171, 1162, 1087, 1044, 946, 890, 847, 828, 793, 725, 656; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{15}H_{25}N_2O_2$ 265.1916, found: 265.1916.

(2*S*,3*S*)3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-3-(4-hydroxyphenyl)propyl]amino]carbonyl]-oxiranecarboxylic acid ethyl ester (42). To a solution of acid **15** (0.12 g, 0.73 mmol) and amine **41** (0.19 mL, 0.73 mmol) in DMF (5 mL) under nitrogen at 23°C was added EEDQ (0.36 g, 1.46 mmol). The resulting mixture was stirred for 7 h. The mixture was then partitioned between H_2O and EtOAc. The organic portion was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (elution with a gradient of hexanes–EtOAc 5:5 to 3:7) to afford 0.14 g (61%) of **42** as a white solid: mp 159°C; $[\alpha]_D=+38.5°$ (c=2.0; $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.03 (m, 2H), 6.77 (m, 2H), 6.59 (d, J=8 Hz, 1H), 5.77 (m, 1H), 4.27 (m, 3H), 3.66 (d, J=2 Hz, 1H), 3.52 (d, J=2 Hz, 1H), 3.26 (m, 2H), 2.59 (m, 2H), 2.10 (m, 1H), 1.95 (m, 1H), 1.59 (m, 1H), 1.38 (q, J=7 Hz, 2H), 1.32 (t, J=7 Hz, 3H), 0.92 (d, J=7.5 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.66, 166.57, 166.23, 154.51, 131.87, 129.34, 115.55, 62.37, 53.66, 52.68, 52.56, 38.15, 38.07, 33.96, 30.74, 25.79, 22.33, 14.00; IR ($CHCl_3$) ν cm^{-1} 3296, 3090, 2957, 1745, 1651, 1617, 1548, 1516, 1445, 1370, 1227, 1204, 1096, 1026, 898, 831; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{21}H_{31}N_2O_6$ 407.2182,: 407.2190. Anal. Calcd for $C_{21}H_{30}N_2O_6$: C, 62.05; h 7.44; N, 6.89. Found: C, 62.03; H, 7.55; N 6.71.

(2*S*,3*S*)-3-[[[(1*R*)-1-[[(3-Methylbutyl)amino]carbonyl]-3-(4-hydroxyphenyl)propyl]amino]carbonyl]-oxiranecarboxylic acid (43). A 1N solution of KOH (0.5 mL, 0.50 mmol) in ethanol was added to a solution of ester **42** (0.10 g, 0.25 mmol) in EtOAc (2 mL) and THF (2 mL) at 0°C. The reaction mixture was allowed to warm to 23°C and stirred for 2 h. The solvent was evaporated in vacuo, the residue dissolved in cold water (10 mL), acidified to pH 2 with 1 M $KHSO_4$ and extracted with EtOAc (5×10 mL). The combined organic portions were washed with brine and dried over Na_2SO_4. Evaporation of the solvent in vacuo afforded 76 mg (81%) of crude **43** as a foam which solidified on standing: mp 68°C; $[\alpha]_D=+70.9°$ (c=3.3; CH_3OH); ^1H NMR (DMSO-d_6) δ 9.15 (s, 1H), 8.67 (d, J=8 Hz, 1H), 7.98 (t, J=6 Hz, 1H), 6.93 (d, J=8 Hz, 2H), 6.65 (d, J=8 Hz, 2H), 4.20 (m, 1H), 3.70 (d, J=1.5 Hz, 1H), 3.50 (d, J=1.5 Hz, 1H), 3.07 (m, 2H), 2.41 (m, 2H), 1.87 (m, 1H), 1.77 (m, 1H), 1.55 (m, 1H), 1.28 (q, J=7 Hz, 2H), 0.86 (d, J=7, 6 Hz); ^{13}C NMR (DMSO-d_6) (100 MHz) δ 170.44, 168.74, 165.12, 155.38, 131.10, 128.97, 115.06, 52.68, 51.15, 37.97, 36.73. 34.09, 30.50, 25.06, 22.32, 22.29; IR (KBr) ν cm^{-1} 3322, 3101, 2958, 2871, 1742, 1654, 1542, 1516, 1451, 1368, 1228, 1015, 852, 897, 830, 762, 655;

HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{19}H_{27}N_2O_6$ 379.1869, found: 379.1864.

(2*S*,3*S*)-*N*-[(1*R*)-1-Phenyl-[[3-(3-methylbutyl)amino]carbonyl]-propyl]-*N′*-(phenylmethoxy)-2,3-oxiranedicarboxamide (45). To a solution of acid **11c** (0.18 g, 0.5 mmol) and *O*-benzyl hydroxylamine **44** (0.06 g, 0.50 mmol, prepared from a solution of its HCl salt by neutralization with $NaHCO_3$ and extraction with EtOAc) in $CHCl_3$ (2 mL) and DMF (2 mL) under nitrogen at 23°C was added EEDQ (0.25 g, 1.00 mmol). The resulting mixture was stirred for 4 h. The mixture was then partitioned between H_2O and EtOAc. The organic portion was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (eluted with CH_2Cl_2–isopropanol 97:3) to afford 0.17 g (50%) of **40** as a white solid. mp: 175°C; $[\alpha]_D$=+32.4° (*c*=2.1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 9.45 (s, 1H), 7.37 (s, 5H), 7.28–7.13 (m, 5H), 6.40 (s, 1H), 4.91 (s, 2H), 4.11 (q, *J*=9 Hz, 1H), 3.56 (d, *J*=2 Hz, 1H), 3.50 (d, *J*=2 Hz, 1H), 3.27 (m, 1H), 3.13 (m,1H), 2.64 (t, *J*=8 Hz, 2H), 2.08 (m, 1H), 1.99 (m, 1H), 1.57 (m, 1H), 1.36 (q, *J*=9 Hz, 2H), 0.90 (d, *J*=8 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.56, 165.80, 163.13, 140.42, 134.66, 129.25, 129.01, 128.70, 128.60, 128.32, 126.33, 78.50, 54.13, 53.18, 52.78, 38.24, 38.05, 33.96, 31.77, 25.85, 22.41, 22.34; IR ($CHCl_3$) ν cm^{-1} 3285, 2956, 1648, 1547, 1498, 1454, 1367, 1232, 1043, 896; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{26}H_{34}N_3O_5$: 468.2498; found: 468.2493

(2*S*,3*S*)-*N*-[(1*S*)-1-Phenyl-[[3-(3-methylbutyl)amino]carbonyl]-propyl]-*N′*-(phenylmethoxy)-2,3-oxiranedicarboxamide (46). Obtained in 51% yield from **12** and **44** by the procedure described above. The solid decomposes at ~100°C without melting: $[\alpha]_D$=+80.0° (*c*=1.0; ethanol); ^{1}H NMR ($CDCl_3$+CD_3OD) δ 7.66 (d, *J*=8 Hz, 1H), 7.33–7.06 (m, 11H), 4.82 (s, 2H), 4.23 (q, *J*=8 Hz, 1H), 3.48 (d, *J*=1.5 Hz, 1H), 3.27 (d, *J*=1.5 Hz, 1H), 3.11(m, 2H), 2.51 (t, *J*=7.5 Hz, 2H), 1.97 (m, 1H), 1.85 (m, 1H), 1.52 (m, 1H), 1.30 (q, *J*=7 Hz, 2H), 0.83 (d, *J*=7 Hz, 6H); ^{13}C NMR ($CDCl_3$+CD_3OD) δ 171.91, 167.24, 164.40, 141.23, 129.65, 129.23, 128.94, 128.74, 126.61, 78.69, 53.82, 53.48, 53.00, 38.46, 38.30, 34.53, 32.24, 30.07, 26.24, 22.57; IR (KBr) ν cm^{-1} 3289, 3088, 3030, 2955, 2927, 2871, 1677, 1661, 1640, 1552, 1498, 1454, 1368, 1230, 1050, 992, 896, 843, 787, 747, 698; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{26}H_{34}N_3O_5$; 468.2498; found: 468.2507.

(2*S*,3*S*)-*N*-[(1*R*)-1-(4-Hydroxyphenyl)-[[3-(3-methylbutyl)-amino]carbonyl]propyl]-*N′*-(phenylmethoxy)-2,3-oxiranedicarboxamide (47). Obtained in 49% yield from **43** and **44** by the procedure described above. mp 162°C; $[\alpha]_D$=+66.35° (*c*=2.0; CH_3OH); ^{1}H NMR (DMSO-d_6) 500 MHz δ 11.68 (s, 1H), 9.15 (s, 1H), 8.70 (d, *J*=8 Hz, 1H), 7.97 (t, *J*=5.5 Hz, 1H), 7.37 (m, 5H), 6.95 (d, *J*=8.5 Hz, 2H), 6.65 (d, *J*=8.5 Hz, 2H), 4.83 (s, 2H), 4.19 (m, 1H), 3.73 (d, *J*=1.5 Hz, 1H), 3.42 (d, *J*=1.5 Hz, 1H), 3.07 (m, 2H), 2.44 (m, 1H), 2.39 (m, 1H), 1.86 (m, 1H), 1.77 (m, 1H), 1.55 (m, 1H), 1.28 (q, *J*=7 Hz, 2H), 0.85 (d, *J*=6 Hz, 6H); ^{13}C NMR (DMSO-d_6) δ 170.37, 165.25, 162.83, 155.32, 135.44, 131.09, 128.88, 128.77, 128.28, 128.23, 115.01, 76.98, 52.57, 52.57, 52.29, 51.17, 37.91, 36.69, 34.07, 30.46, 25.01, 22.24, 22.20; IR (KBr) ν cm^{-1} 3315, 3209, 3030, 2957, 1702, 1677, 1654, 1560, 1516, 1457, 1368, 1228, 1043, 990, 896, 832, 751, 699; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{26}H_{34}N_3O_6$ 484.2447, found: 484.2437. Anal. Calcd for $C_{26}H_{33}N_3O_6$: C, 64.58, H, 6.88; N, 8.69; found: C, 64.24; H, 6.98; N, 8.61.

(2*S*,3*S*)-*N*-[(1*R*)-1-(4-Hydroxyphenyl)-[[3-phenylamino]-carbonyl]-propyl]-*N′*-(phenylmethoxy)-2,3-oxiranedicarboxamide (48). Obtained in 53% yield from **38** and **44** by the procedure described above: mp 170°C (dec); $[\alpha]_D$=+43.8° (*c*=2.1; CH_3OH); ^{1}H NMR (DMSO-d_6) δ 11.73 (d, *J*=3 Hz, 1H), 10.14 (s, 1H), 8.95, (d, *J*=5.5 Hz, 1H), 7.59 (d, *J*=6.5 Hz, 2H), 7.40 (m, 4H), 7.23 (m, 4H), 7.20 (d, *J*=6 Hz, 2H), 7.06 (m, 1H), 4.84 (d, *J*=3 Hz, 1H), 4.46 (m, 1H), 3.76 (s, 1H), 3.46 (s, 1H), 2.68 (m, 1H), 2.58 (m, 1H), 2.04 (m, 1H) 1.96 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 169.78, 165.64, 162.80, 140.94, 138.68, 135.50, 128.87, 128.66, 128.32, 128.18, 125.90, 123.46, 119.43, 77.05, 53.51, 52.26, 51.23, 33.52, 31.54; IR (KBr) ν cm^{-1} 3287, 3046, 2924, 1679, 1653, 1600, 1546, 1498, 1447, 1383, 1316, 1245, 1042, 1007, 969, 898, 754, 700; HRMS, FAB $[M+Na]^+$ calcd for $C_{27}H_{27}N_3O_6Na$ 496.1848, found: 496.1858.

(2*S*,3*S*)-*N*-[(1*R*)-1-Phenyl-[[3-(3-methylbutyl)amino]carbonyl]-propyl]-*N′*-(2-phenylethyl)-2,3-oxiranedicarboxamide (49). To a solution of **11c** (0.100 g, 0.276 mmol), Phenethylamine (0.035 mL, 1.33 mmol), *N*-methylmorpholine (0.035 mL, 0.276 mmol) and HOBT (0.042 g, 0.276 mmol) in $CHCl_3$ (4 mL) under nitrogen at 0°C was added EDC (0.0.58 g, 0.306 mmol). The resulting mixture was stirred for 1 h at this temperature, then at 23°C for 10 h. The mixture was then partitioned between H_2O and a mixture of EtOAc–hexanes 4:1. The organic portion was washed with brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (elution with hexanes–EtOAc 7:3) to afford 0.128 g (80%) of **49** as a white solid: mp 174–176°C; $[\alpha]_D$=+26.4° (*c*=2.2; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.35–7.16 (m, 10H), 6.55 (d, *J*=8 Hz, 1H), 6.01 (t, *J*=7 Hz, 1H), 5.71 (t, *J*=5.5 Hz, 1H), 4.31 (q, *J*=7 Hz, 1H), 3.54 (m, 2H), 3.46 (d, *J*=1.5 Hz, 1H), 3.32 (d, *J*=1.5 Hz, 1H), 3.26 (m, 2H), 2.83 (t, *J*=7 Hz, 2H), 2.65 (m, 2H), 2.14 (m, 1H), 1.97 (m, 1H), 1.60 (m, 1H), 1.38 (q, *J*=7 Hz, 2H), 0.92 (d, *J*=7 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 170.50, 165.89, 165.67, 140.49, 138.22, 128.66, 128.51, 128.26, 126.67, 126.19, 54.47, 52.63, 40.19, 38.15, 37.92, 35.30, 34.03, 31.73, 25.77, 22.39, 22.31; IR ($CHCl_3$) ν cm^{-1} 3290, 3088, 2956, 1651, 1551, 1498, 1455, 1367, 1236, 1085, 1031, 895, 747, 698; HRMS, CI (NH_3) $[M+H]^+$ calcd for $C_{27}H_{36}N_3O_4$: 466.2705, found: 466.2691.

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