Pergamon

Tetrahedron, Vol. 53, No. 14, pp. 5325-5338, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(97)00195-6

Functionalized *Erythro* *N*-Protected α-Amino Epoxides. Stereocontrolled Synthesis and Biological Activity

Amnon Albeck* and Gary I. Estreicher

Department of Chemistry, Bar Ilan University, Ramat Gan 52900, Israel

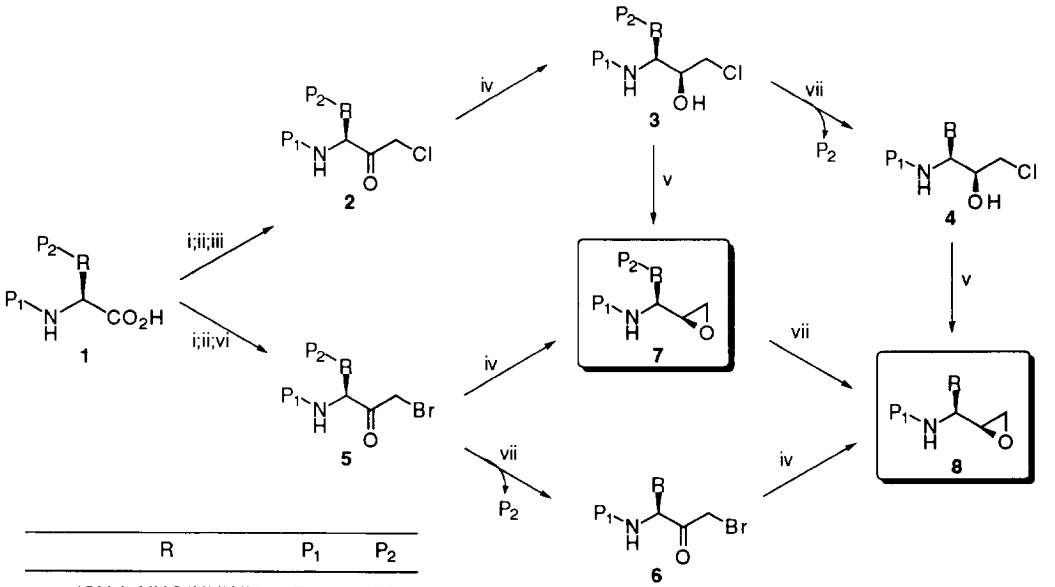
Abstract: *Erythro* *N*-protected α-amino epoxides, derived from α-amino acids bearing functionalized side chains, were synthesized. The key synthetic step is a stereoselective reduction of the corresponding haloketone either to the halohydrin or directly to the epoxide. The side chains include ester, ether and alcohol, nitro guanidine, carbamate and amine functional groups, derived from aspartate and glutamate, serine, arginine, and lysine, respectively. The epoxides derived from lysine and N_{ω}–nitro-arginine exhibited selective inactivation of the cysteine proteases papain and cathepsin B, while they failed to inactivate the serine protease trypsin. © 1997 Elsevier Science Ltd.

INTRODUCTION

α-Amino epoxides can serve as versatile chiral synthetic building blocks, since they undergo regio- and stereoselective attack by various nucleophiles.[1,2] They were extensively used for the synthesis of hydroxyethylene dipeptide isosters,[3] anti cancer and antibiotic natural products,[4] novel amino acids,[5] NMDA antagonists,[6] and others. α-Amino epoxides and peptidyl epoxides are also selective cysteine protease inactivators, while exhibiting no inhibitory activity towards serine proteases.[7] A variety of approaches towards stereoselective synthesis of this family of compounds were recently developed. They include epoxidation of α-amino aldehydes by sulfonium ylides,[8] epoxidation of allyl amines,[9] and reduction of *N,N*-dibenzyl-α-amino chloromethyl ketones[10] to stereoselectively synthesize *threo* α-amino epoxides or peptidyl epoxides. On the other hand, reduction of *N*-protected-α-amino halomethyl ketones[3h,11] or peptidyl bromomethanes,[11a] condensation of dihalomethane with *N,N*-dibenzyl-α-amino aldehydes,[10] cyclization of 3-amino-1,2-diols,[12] epoxidation of *N,N*-doubly protected α-amino aldehydes by sulfonium ylides,[13] and reductive amination of α-keto epoxides[14] yield preferentially the corresponding *erythro* α-amino- or peptidyl epoxides. These synthetic routes were applied almost exclusively to hydrophobic α-amino epoxides, bearing only simple alkyl or aryl side chains, either because they satisfied some specific requirements or in order to avoid synthetic complications.[15] Nevertheless, functionalized α-amino epoxides can serve as useful synthetic building blocks. Furthermore, many proteases (e.g. trypsin,[16] papain,[17] cathepsin B,[17a] interleukin 1β converting enzyme,[18] and the 3C protease of human rhinovirus[19]) preferentially hydrolyze amide bonds at amino acids bearing functionalized side chains. Therefore, inhibitors based on such amino acids are of interest. Thus, synthesis of highly functionalized α-amino epoxides remained an important synthetic target. In this paper we describe some of the scopes and limitations in applying one of the above approaches, namely stereoselective reduction of α-amino halomethyl ketones,[11a] towards the stereoselective synthesis of such highly functionalized *erythro* α-amino epoxides. We also demonstrate utilization of such compounds in selective inactivation of cysteine proteases.

RESULTS AND DISCUSSION

An efficient general approach for the synthesis of *erythro N*-protected α-amino epoxides, recently developed in our laboratory, is based on a key stereoselective reduction of α-amino haloketones.[11a] It utilizes the chirality of α-amino acids to induce a preferred configuration at the new adjacent chiral center. The fate of the reduction depends very much on the halide, chloroketones being reduced to the corresponding chlorohydrins and bromoketones being reduced and spontaneously cyclized to the corresponding epoxides under the reaction conditions. The haloketone itself can be synthesized in a three-step-one-pot reaction from the corresponding α-amino acid via a mixed anhydride and a diazoketone. In the present study, α-amino acids bearing a protected functional group at their side chains were subjected to the same procedure, yielding the corresponding doubly protected α-amino epoxides. In addition, removal of the side chain protecting group either prior or subsequent to the epoxide formation was also carried out. This general approach is summarized in Scheme 1. The α-amino acids that were studied are arginine, lysine, serine, aspartic acid and glutamic acid. Their side chain functional groups contain guanidine, amine, alcohol, and carboxylic acid, protected as nitro guanidine, carbamate, ether[20] and ester, respectively.



	R	P_1	P_2
a	$(CH_2)_3NHC(NH)NH$	Boc	NO_2
b	$(CH_2)_4NH$	Cbz	Boc
c	CH_2O	Boc	Bn
d	$(CH_2)_2CO_2$	Cbz	*t*-Bu
e	CH_2CO_2	Cbz	*t*-Bu
f	CH_2CO_2	Boc	Bn

[a] Key: (i) $ClCO_2CH_2CHMe_2$; NMM, THF; (ii) CH_2N_2, Et_2O; (iii) HCl (g); (iv) $NaBH_4$, EtOH; (v) NaOMe, MeOH; (vi) HBr (aq); (vii) selective deprotection: for a,c,f - 1,4-cyclohexadiene, Pd/C, EtOH; for b,d,e - TFA, CH_2Cl_2.

Scheme 1. General synthetic approach to functionalized *erythro* α-amino epoxides

Synthesis of fully protected α-amino epoxides

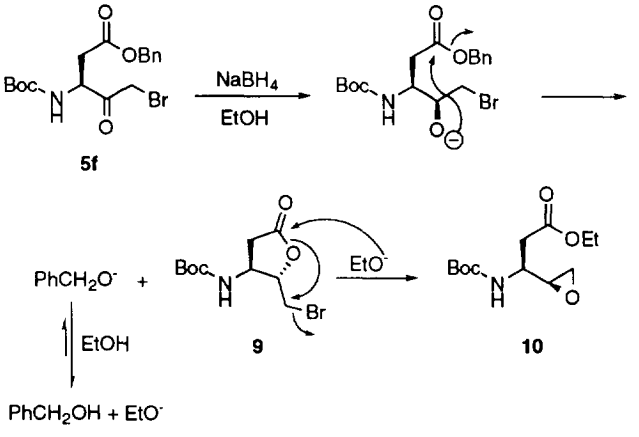
Both routes, via the chloroketone and the bromoketone, were studied since they enable removal of the side chain protecting groups at two different stages along the synthesis. The doubly protected chloroketones **2** and bromoketones **5** were synthesized in very good to excellent yields (87-100%, with only one exception, the bromoketone derived from nitro arginine which was obtained in 47% yield).

Bromoketones **5** were reduced by $NaBH_4$, yielding the corresponding epoxides **7** directly. The reduction proceeded in good yield (62-99%) and stereoselectivity (66-80% *de*) (Table 1). One exception was the attempt to reduce *N*-Boc-*O*-Bn-aspartyl bromomethane **5f**. The expected epoxide **7f** could not be isolated. Instead, benzyl alcohol, a small amount of *N*-Boc-aspartyl bromomethyl lactone **9** (identified by its HRMS), *N*-Boc-*O*-ethyl aspartate epoxide (**10**, 11% yield), and a few other uncharacterized products were isolated. This can be explained by assuming a favorable competing reaction in which the intermediate alkoxide attacks the benzyl ester to form the lactone. The latter can be solvolyzed by EtOH and trapped as the corresponding epoxide (Scheme 2).

Table 1. Yield[a] and Product Ratio[b] for the Stereoselective Synthesis of Doubly Protected α-Amino Epoxides **7** from Bromomethyl Ketones **5**

epoxide	yield (%)	*erythro:threo*
7a	62	6.0 : 1
7b	85	4.9 : 1
7c	97	6.5 : 1
7d	99	9.4 : 1
7e	92	6.1 : 1

[a] yields from the corresponding bromomethyl ketone **5**.
[b] determined by ^1H NMR.



Scheme 2. Chemical transformations of protected aspartyl bromoketone **5f**

The chloroketones derived from lysine (**2b**) and from glutamate (**2d**) were also reduced by $NaBH_4$, yielding the corresponding chlorohydrins **3b** and **3d** accompanied by small amounts of the corresponding epoxides **7b** and **7d** (24% yield, 3:1 *erythro:threo* ratio for the latter). This observation was previously noted also for the simple "unfunctionalized" side chain chloroketones.[11a] Chlorohydrin **3b** was transferred to α-amino epoxide **7b** by treatment with one equivalent of NaOMe in 53% yield and a 4.9:1 *erythro:threo* ratio. Comparison of the two routes to **7b** clearly demonstrates that in addition to being shorter, the bromoketone route is superior to the chloroketone route also in the overall yield of the epoxide product (76% and 46% yield, respectively).

Epoxides **7** can then serve as highly functionalized synthetic building blocks, being reacted with various nucleophiles at the epoxidic moiety, followed by further manipulations of the amino group, the alcohol or the side chain functional group after an appropriate selective deprotection step.

Removal of side chain protecting groups

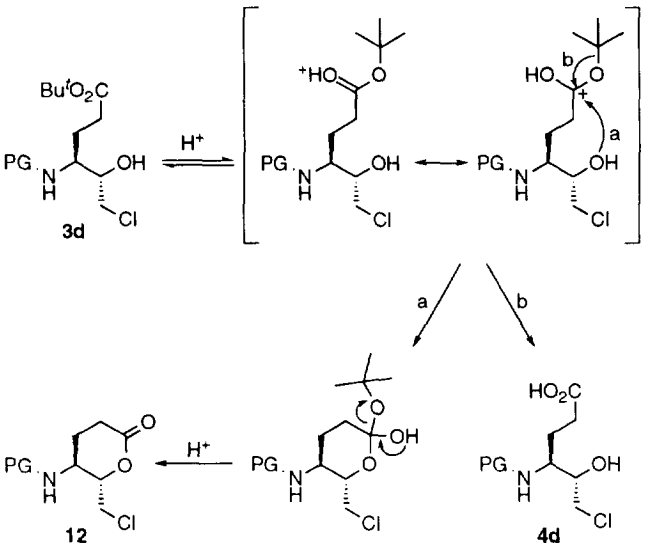
The side chain protecting groups could be selectively removed in the presence of the protected amino groups. In principle, they can be removed at three different stages; from bromoketones **5**, from chlorohydrins **3**, and from the protected epoxides **7**. All three possibilities were explored in the present study.

Acidic deprotection of the *N*-Boc and *O*-*t*-butyl protecting groups of the bromoketone derivatives of lysine, glutamate and aspartate (**5b**, **5d** and **5e**, respectively) produced the corresponding unprotected bromoketones **6** in excellent yields (93-99%). However, $NaBH_4$ reduction of **6**, in analogy to the corresponding reduction of the doubly protected bromoketones **5**, did not always yield the expected product. The lysyl bromomethane **6b** yielded the desired *erythro* α-amino epoxide **8b** bearing an unprotected side chain as a single isomer. The *threo* isomer could not be detected by either ^1H or ^{13}C NMR. On the other hand, the glutamyl bromomethane **6d** was reduced to the corresponding bromohydrin **11** in a 2.9:1 *erythro:threo* ratio (as determined by ^1H NMR), while attempts to reduce the aspartate analog **6e** did not yield any identifiable product.

Similar observations were also made upon deprotection of the chlorohydrins **3b** and **3d**, derived from lysine and glutamate, respectively. Treatment of **3d** with TFA afforded the corresponding free acid **4d** in low yield (38%), along with the corresponding δ-lactone **12** (identified by its HRMS) (Scheme 3). On the other hand, deprotection of the lysine derivative **3b** afforded **4b** in quantitative yield. Treatment of **4b** with one equivalent of a base produced the target epoxide **8b** in quantitative yield. Only the *erythro* isomer could be detected by ^1H and ^{13}C NMR.

Removal of the side chain protecting group of the serine epoxide **7c** by catalytic transfer hydrogenation[21] afforded the unprotected α-amino epoxide **8c** in very poor yield (14%).

The stereoselectivity of the $NaBH_4$ reduction of *N*-protected-α-amino bromomethyl ketones, yielding preferentially the *erythro* relative configuration is well established.[3h,11] It was previously noted that the two stereoisomers, *erythro* and *threo*, of α-amino epoxides could be distinguished by the epoxidic region of their ^1H NMR spectra. While the *erythro* isomer displays a "compact" set of resonances, the *threo* isomer has a more "spread out" and better resolved spectrum.[11a] The side chain functionalized α-amino epoxides described in this study are no exception, as is clearly seen from Table 2.



Scheme 3. Chemical transformations of protected glutamate chlorohydrin **3d**

Table 2. ^{1}H NMR Chemical Shifts[a,b] (in ppm) of Epoxidic Protons.

α-amino epoxide	*erythro*			*threo*		
	H_1	H_t	H_c	H_1	H_t	H_c
7a[c]	2.86	2.65	2.67	2.99	2.55	2.69
7b	2.87	2.73 - 2.80		3.01	2.58	2.72
7c	3.09	2.76 - 2.78		3.22	2.63	2.75
7d	2.89	2.78	2.78	3.03	2.57	2.72
7e	3.07	2.77	2.79	3.15	2.60	2.74
8b[d]	2.86	2.67	2.68	---	---	---
8c	2.98	2.69	2.69	3.12	2.55	2.69
10	3.08	2.74	2.81	3.13	2.58 - 2.78[e]	

[a] In $CDCl_3$, unless otherwise stated. [b] The *cis-trans* assignment is based on the vicinal J coupling.[22] [c] In acetone-d_6. [d] In CD_3OD. The *threo* isomer was not detected in the spectrum. [e] obscured.

Biological activity

It was previously demonstrated that *erythro* *N*-protected α-amino epoxides and peptidyl epoxides are selective inactivators of cysteine proteases, while exhibiting no activity towards serine proteases.[7] However, only peptidyl epoxides derived from hydrophobic (non-functionalized) side-chain amino acids were used in those studies. In order to generalize and extend this observed selectivity also to enzymes that catalyze hydrolysis of amide bonds of functionalized side chain P_1 amino acids,[23] the α-amino epoxides derived from lysine and arginine were examined as irreversible inactivators of the serine protease trypsin and the cysteine proteases cathepsin B and papain. Neither N_α-Cbz-Lys-epoxide (**8b**) nor N_α-Boc-N_ω-nitro-Arg-epoxide (**7a**) inhibited the serine protease trypsin, even after 24 h incubation with 5 mM inhibitor. However, as predicted, the same α-amino epoxides exhibited time- and concentration-dependent inactivation of the two cysteine proteases. One such graphical presentation of the inactivation process (a semilog plot of the residual enzymatic activity as a function of incubation time with the inactivator, at various inactivator concentrations) is shown in Figure 1A.

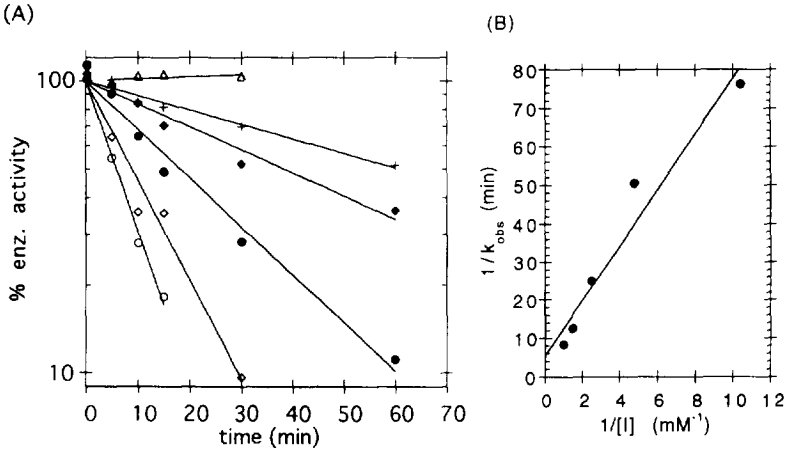


Figure 1. (A) Time course of the inactivation of the cysteine protease papain by N_α-Cbz-Lys-epoxide **8b**, in 100 mM phosphate buffer containing 0.5 mM DTT and 2 mM EDTA, at 25 °C. Inactivator concentration: 0.0 (△), 0.1 (+), 0.2 (♦), 0.4 (•), 0.7 (◊) and 1.0 (○) mM. (B) Kitz-Wilson plot of $1/K_{obs}$ vs. $1/[I]$.

The kinetic data were processed to fit the following minimal kinetic scheme, corresponding to the expected inactivation mechanism[7b,c]:

$$E + I \underset{}{\overset{K_i}{\rightleftharpoons}} E{\cdot}I \xrightarrow{k_i} E\text{-}I$$

The kinetic parameters of the inactivation process K_i *and* k_i (Table 3, Figure 1B) were derived according to the equation[24]

$$1/k_{obs} = (K_i/k_i)(1/[I]) + 1/k_i$$

where k_{obs} is the observed first order rate constant of inactivation ($=\ln 2/t_{1/2}$) for each initial inactivator concentration [I], K_i is the apparent dissociation constant of the reversible enzyme-inactivator complex, and k_i is the limiting first order rate constant of the inactivation reaction (from the complex).

Table 3. Kinetic parameters for the inactivation of the cysteine proteases cathepsin B and papain by α-amino epoxides.

epoxide	cathepsin B			papain		
	k_i (min^{-1})	K_i (mM)	k_i/K_i ($M^{-1}s^{-1}$)	k_i (min^{-1})	K_i (mM)	k_i/K_i ($M^{-1}s^{-1}$)
7a	0.045	1.48	0.52	--	--	0.10[a]
8b	--	--	0.34[a]	0.125	0.94	2.22

[a] Measurements were limited to the linear range, where $[I] \leq K_i$. Therefore, only 2nd order rate constant was obtained.

It should be noted that lysine- and arginine-based chloromethanes[25] and fluoroalkanes,[26] and the natural product family of leupeptins (peptidyl argininals)[27] all inhibit trypsin, in accordance with its known primary specificity. Chloromethyl ketones, diazomethyl ketones, and aldehydes derived from lysine and arginine (including the natural products leupeptins) are also good inhibitors of cathepsin B and papain.[17a,25,27] Therefore, it was expected that the α-amino epoxide derived from lysine would bind in the active site of all of these enzymes. Thus, the selectivity between serine and cysteine proteases, exhibited by the peptidyl epoxide derived from lysine, is interpreted as stemming from a subtle difference in the catalytic mechanism of the two protease families.[7b,c,28] In addition, the results presented here (Table 3) also indicate that the peptidyl epoxides can selectively inhibit different enzymes within the family of cysteine proteases, based on the substrate specificity of these enzymes. Cbz-Lys-epoxide inactivates papain an order of magnitude faster than it inactivates cathepsin B, while the nitro arginine derived epoxide is a better inhibitor of cathepsin B and it exhibits only very poor inactivation of papain. The relative potency of the two inhibitors, as well as the dipeptidyl epoxides previously described by us,[7c] towards cathepsin B parallels the selectivity exhibited by other α-amino acid based inhibitors such as chloromethyl ketones and diazomethyl ketones.[17a]

SUMMARY

α-Amino epoxides are attractive synthetic building blocks containing at least two well defined chiral centers. The present study expands and generalizes a synthetic method based on a stereoselective reduction of α-amino haloketones to "simple" α-amino epoxides by applying it also to α-amino epoxides bearing a variety of functional groups at their side chains. These include esters, ether, alcohol, nitro guanidine, carbamate and primary amine. The scope and limitations of this approach were explored.

We further demonstrated utilization of these functionalized α-amino epoxides as selective cysteine protease inactivators, exhibiting selectivity between serine- and cysteine proteases and within the latter family.

EXPERIMENTAL SECTION

General. ^1H and ^{13}C NMR spectra were recorded at 300 or 200 MHz and 75 or 50 MHz, respectively, in $CDCl_3$, unless otherwise specified. Chemical shifts are reported in ppm relative to TMS in $CDCl_3$ or relative to solvent resonance in other solvents. All ^1H NMR assignments were supported by homonuclear decoupling experiments, while ^{13}C NMR assignments were supported by off-resonance heteronuclear decoupling experiments. Mass spectra were recorded in CI mode with methane as the reagent gas, unless otherwise indicated. TLC was performed on E. Merck 0.2 mm precoated silica gel F-254 plates, and viewed by Cl_2/KI-tolidine[29]. Flash column chromatography[30] was carried out on silica gel 60 (230-400 mesh ASTM, E. Merck). Amino acids, enzymes and their substrates were purchased from Sigma Chemical Company. Anhydrous solvents were dried and freshly distilled (THF and ether from sodium/benzophenone, and CH_2Cl_2 from 4Å molecular sieves). Diastereomeric (*erythro:threo*) ratios were determined from the relevant ^1H NMR spectra of the crude mixtures.

Doubly-Protected α-amino chloromethyl ketones (2), α-amino chlorohydrins (3), α-amino bromomethyl ketones (5) and **α-amino epoxides (7)** were synthesized according to a published procedure.[11a]

***N_α*-Cbz-*N_ε*-Boc-lysyl chloromethane (2b)**: 99% yield. ^1H NMR 7.347 (s, 5H), 5.683 (d, J=7.0 Hz, 1H), 5.099 (s, 2H), 4.627 (bt, 1H), 4.555 (dt, J=4.4, 7.8 Hz, 1H), 4.273 (s, 2H), 3.099 (q, J=5.4 Hz, 2H), 1.85 (m, 1H), 1.66 (m, 1H), 1.415 (s, 9H), 1.55-1.30 (m, 4H); ^{13}C NMR 201.35, 156.21, 156.15, 136.02, 128.52, 128.26, 128.12, 79.30, 67.21, 57.72, 46.40, 39.56, 28.37, 30.72, 29.68, 22.13; MS *m/z* 415, 413 (12, 36, MH^+), 359, 357 (7, 18), 315, 313 (35, 100), 279 (8), 277 (11), 259 (45), 91 (29); HRMS calcd for $C_{20}H_{30}N_2O_5{}^{35}Cl^+$ 413.1843, found 413.1945.

***N*-Cbz-*O-t*-butyl-glutamyl chloromethane (2d)**: 98% yield. ^1H NMR 7.314 (s, 5H), 5.926 (d, J=7.8 Hz, 1H), 5.076 (s, 2H), 4.550 (td, J=8.0, 4.1 Hz, 1H), 4.309 (s, 2H), 2.328 (dt, J=16.7, 7.4 Hz, 1H), 2.280 (dt, J=16.7, 6.4 Hz, 1H), 2.23-2.02 (m, 1H), 1.840 (dq, J=14.5, 7.1 Hz, 1H), 1.415 (s, 9H); ^{13}C NMR 200.66, 171.84, 155.95, 135.88, 128.31, 128.00, 127.83, 80.79, 66.93, 57.11, 46.31, 30.74, 27.79, 25.86; MS *m/z* 406, 404 (7, 21, MH^++34), 372, 370 (19, 53, MH^+), 316, 314 (35, 100), 272, 270 (16, 51), 236 (14); HRMS calcd for $C_{18}H_{25}NO_5{}^{35}Cl^+$ 370.1421, found 370.1642; calcd for $C_{18}H_{25}NO_5{}^{37}Cl^+$ 372.1578, found 372.1699.

***N_α*-Cbz-*N_ε*-Boc-lysine chlorohydrin (3b)**: 87% yield, after flash chromatography (CH_2Cl_2:EtOAc 5:1). ^1H NMR 7.345 (s, 5H), 5.154 (d, J=8.9 Hz, 1H), 5.091 (s, 2H), 4.630 (bt, 1H), 3.85-3.68 (m, 2H, CHα), 3.616 (dd, J=11.2, 3.8 Hz, 1H), 3.531 (dd, J=11.4, 7.5 Hz, 1H), 3.235 (bs, 1H), 3.080 (bt, 2H), 1.70-1.58 (m, 2H), 1.417 (s, 9H), 1.57-1.40 (ob, 4H); ^{13}C NMR 156.57, 156.14, 136.31, 128.50, 128.16, 128.06, 79.21, 74.23, 67.03, 53.87, 47.14, 40.10, 28.43, 29.82, 29.23, 22.80; MS *m/z* 417, 415 (0.3, 1.3, MH^+), 345, 343 (4, 11), 323 (12), 317, 315 (30, 100), 299, 297 (2, 5), 279 (24); HRMS calcd for $C_{20}H_{32}NO_5{}^{35}Cl^+$ 415.2000, found 415.1942.

***N*-Cbz-*O-t*-butyl-glutamate chlorohydrin (3d)**: 50% yield (accompanied by 24% yield of the corresponding epoxide **7d**), after flash chromatography (CH_2Cl_2:EtOAc 19:1). ^1H NMR 7.301 (s, 5H), 5.366 (d, J=8.9 Hz, 1H), 5.059 (s, 2H), 3.86-3.65 (m, 2H), 3.589 (dd, J=11.2, 3.9 Hz, 1H), 3.518 (dd, J=11.4, 7.4 Hz, 1H), 3.423 (d, J=4.3 Hz, 1H), 2.294 (t, J=7.2 Hz, 2H), 1.901 (dtd, J=14.3, 7.4, 3.4 Hz, 1H), 1.732 (ddt, J=15, 8, 6 Hz, 1H), 1.395 (s, 9H); ^{13}C NMR 172.81, 156.40, 136.23, 128.41, 128.05, 127.94, 80.59,

74.08, 66.86, 53.46, 46.88, 31.80, 27.94, 24.42; MS *m/z* 374, 372 (5, 16, MH^+), 336 (14), 318, 316 (32, 100), 280 (27), 274, 272 (21, 61), 236 (30), 91 (34); HRMS calcd for $C_{18}H_{27}NO_5{}^{35}Cl^+$ 372.1578, found 372.1563; calcd for $C_{18}H_{27}NO_5{}^{37}Cl^+$ 374.1548, found 374.1549.

N_α-Boc-N_ω-Nitro-arginyl bromomethane (5a): 47% yield, after flash chromatography (CH_2Cl_2:EtOAc 5:1→3:1). ^1H NMR 8.694 (bs, 1H), 7.720 (bs, 2H), 5.784 (d, J=7.6 Hz, 1H), 4.560 (m, 1H), 4.21 (d, J=15 Hz, 1H), 4.19 (d, J=15 Hz, 1H), 3.374 (m, 2H), 2.05-1.50 (m, 4H), 1.432 (s, 9H); ^{13}C NMR 201.41, 159.16, 156.06, 80.54, 56.78, 40.57, 32.46, 28.16, 24.61; MS *m/z* 398, 396 (7, 6, MH^+), 334 (58), 316 (100), 278 (49), 260 (51), 234 (41), 217 (29), 216 (31), 199 (47), 159 (48), 157 (28), 136, 134 (51, 53), 118 (27).

N_α-Cbz-N_ε-Boc-lysyl bromomethane (5b): 89% yield, after flash chromatography (ether:hexane 1:1). ^1H NMR 7.334 (s, 5H), 5.880 (d, J=6.2 Hz, 1H), 5.091 (s, 2H), 4.747 (t, J=5.5 Hz, 1H), 4.556 (dt, J=4.4, 7.9 Hz, 1H), 4.100 (d, J=13.6 Hz, 1H), 4.056 (d, J=13.7 Hz, 1H), 3.079 (q, J=5.9 Hz, 2H), 1.85 (m, 1H), 1.656 (quin., J=6.9 Hz, 1H), 1.409 (s, 9H), 1.55-1.30 (m, 4H); ^{13}C NMR 200.80, 156.17, 136.04, 128.50, 128.22, 128.09, 79.25, 67.18, 57.88, 39.64, 28.36, 31.87, 30.94, 29.64, 22.19; MS *m/z* 405 (20), 359, 357 (98, 100, MH^+-$CO_2{}^t$Bu), 279 (47), 277 (13), 128 (13), 126 (15), 91 (57); HRMS calcd for $C_{15}H_{22}N_2O_3{}^{79}Br^+$ 357.0814, found 357.0810; calcd for $C_{15}H_{22}N_2O_3{}^{81}Br^+$ 359.0793, found 359.0790.

***N*-Boc-*O*-benzyl-seryl bromomethane (5c)**: 87% yield. ^1H NMR 7.313 (s, 5H), 5.569 (d, J=7.5 Hz, 1H), 4.653 (dt, J=7.6, 3.9 Hz, 1H), 4.498 (d, J=12.2 Hz, 1H), 4.463 (d, J=11.9 Hz, 1H), 4.112 (d, J=14.0 Hz, 1H), 4.085 (d, J=14.0 Hz, 1H), 3.886 (dd, J=9.5, 3.9 Hz, 1H), 3.635 (dd, J=9.7, 4.5 Hz, 1H), 1.439 (s, 9H); ^{13}C NMR 199.33, 155.16, 136.89, 128.32, 127.82, 127.55, 80.11, 73.25, 69.29, 57.82 , 32.89, 28.08; MS *m/z* 374, 372 (2, 3, MH^+), 318, 316, (21, 22), 274, 272 (18, 18), 238 (20), 194 (36), 91 (100); HRMS calcd for $C_{16}H_{23}NO_4{}^{79}Br^+$ 372.0810, found 372.0782; calcd for $C_{16}H_{23}NO_4{}^{81}Br^+$ 374.0790, found 374.0779.

***N*-Cbz-*O*-*t*-butyl glutamyl bromomethane (5d)**: 100% yield. ^1H NMR 7.313 (s, 5H), 5.950 (d, J=7.9 Hz, 1H), 5.077 (s, 2H), 4.625 (td, J=7.9, 4.5 Hz, 1H), 4.121 (d, J=13.6 Hz, 1H), 4.090 (d, J=13.5 Hz, 1H), 2.337 (dt, J=16.8, 7.2 Hz, 1H), 2.282 (dt, J=16.8, 6.6 Hz, 1H), 2.25-2.10 (m, 1H), 1.873 (dq, J=14.3, 7.2 Hz, 1H), 1.415 (s, 9H); ^{13}C NMR 200.13, 171.83, 155.89, 135.87, 128.28, 127.97, 127.81, 80.72, 66.89, 57.14, 31.98, 30.74, 27.79, 26.05; MS *m/z* 416, 414 (15, 15, MH^+), 360, 358 (36, 35), 316, 314 (34, 32), 236 (25), 234 (36), 115 (35), 91, (100); HRMS calcd for $C_{18}H_{25}NO_5{}^{79}Br^+$ 414.0916, found 414.1071; calcd for $C_{18}H_{25}NO_5{}^{81}Br^+$ 416.0896, found 416.0941.

***N*-Cbz-*O*-*t*-butyl aspartyl bromomethane (5e)**: 100% yield. ^1H NMR 7.322 (s, 5H), 6.145 (d, J=8.7 Hz, 1H), 5.107 (s, 2H), 4.729 (dt, J=8.7, 5.1 Hz, 1H), 4.180 (s, 2H), 2.853 (dd, J=17.0, 5.2 Hz, 1H), 2.755 (dd, J=17.0, 5.2 Hz, 1H), 1.395 (s, 9H); ^{13}C NMR 199.21, 169.87, 155.74, 135.76, 128.29, 128.03, 127.85, 81.72, 67.06, 54.48, 36.75, 32.38, 27.68; MS *m/z* 436, 434 (17, 17, MH^++34), 402, 400 (24, 24, MH^+), 392, 390 (25, 25), 346, 344 (57, 58), 302, 300 (17, 18), 278 (71), 222 (70), 178 (100), 107 (31), 91 (21); HRMS calcd for $C_{17}H_{23}NO_5{}^{79}Br^+$ 400.0760, found 400.0734; calcd for $C_{17}H_{23}NO_5{}^{81}Br^+$ 402.0739, found 402.0752.

***N*-Boc-*O*-benzyl aspartyl bromomethane (5f)**: 94% yield. ^1H NMR 7.326 (s, 5H), 5.766 (d, J=8.7 Hz, 1H), 5.097 (s, 2H), 4.708 (dt, J=8.6, 5.2 Hz, 1H), 4.190 (s, 2H), 2.999 (dd, J=17.3, 5.2 Hz, 1H), 2.879 (dd, J=17.2, 5.2 Hz, 1H), 1.444 (s, 9H); ^{13}C NMR 199.65, 170.88, 155.10, 135.07, 128.40, 128.23, 128.02, 80.50, 66.76 53.95, 35.59, 32.31, 28.06; MS *m/z* 436, 434 (18, 19, MH^++34), 402, 400

(29, 29, MH^+), 392, 390 (15, 15), 346, 344 (65, 64), 302, 300 (36, 36), 279 (31), 178 (24), 91 (100); HRMS calcd for $C_{17}H_{23}NO_5{}^{79}Br^+$ 400.0760, found 400.0714; calcd for $C_{17}H_{23}NO_5{}^{81}Br^+$ 402.0739, found 402.0701.

N_α-Boc-N_ω-Nitro-arginine epoxide (7a): 62% yield from the corresponding bromomethyl ketone **5a**. *Erythro:threo* 6:1. ^1H NMR (in acetone-d_6) *Erythro*: 7.641 (bs, 3H), 6.113 (d, J=8.3 Hz, 1H), 3.47-3.24 (m, 3H), 2.860 (ddd, J=6.6, 3.5, 3.0 Hz, 1H), 2.672 (dd, J=5.3, 2.7 Hz, 1H), 2.651 (dd, J=5.3, 2.7 Hz, 1H), 1.56-1.88 (m, 4H), 1.395 (s, 9H); *Threo*: 3.24-3.47 (ob, 1H), 2.990 (ddd, J=6.6, 4.3, 2.6 Hz, 1H), 2.688 (dd, J=5.0, 4.0 Hz, 1H), 2.546 (dd, J=5.3, 2.7 Hz, 1H); ^{13}C NMR *Erythro*: 160.93, 156.57, 78.97, 54.42, 52.93, 45.85, 41.67, 28.56, 26.08; *Threo*: 54.11, 52.21, 41.44; MS *m/z* 318 (9, MH^+), 278 (15), 262 (60), 234 (7), 218 (6), 201 (7), 200 (31), 175 (8), 99 (19), 59 (60), 57 (100). HRMS calcd for $C_{12}H_{24}N_5O_5^+$ 318.1777, found 318.1781.

N_α-Cbz-N_ε-Boc-lysine epoxide (7b): 85% yield from the corresponding bromomethyl ketone **5b**. *Erythro:threo* 4.9:1. 53% yield from the corresponding chlorohydrin **3b**. *Erythro:threo* 4.9:1. After flash chromatography (ether:hexane 1:1). ^1H NMR *Erythro*: 7.348 (s, 5H), 5.093 (s, 2H), 4.920 (d, J=7.6 Hz, 1H), 4.593 (t, J=7.7 Hz, 1H), 3.54-3.38 (m, 1H), 3.18-3.03 (m, 2H), 2.869 (dt, J=6.2, 3.1 Hz, 1H), 2.80-2.73 (m, 2H), 1.70-1.40 (m, 6H), 1.419 (s, 9H); *Threo*: 3.04-2.98 (m, 1H), 2.718 (dd, J=4.6, 4.0 Hz, 1H), 2.575 (dd, J=4.6, 2.7 Hz, 1H); ^{13}C NMR *Erythro*: 156.12, 136.32, 128.49, 128.14, 128.09, 79.15, 66.88, 53.94, 52.70, 46.20, 39.94, 28.42, 31.20, 29.87, 22.52; *Threo*: 53.40, 50.45, 44.20; MS *m/z* 379 (71, MH^+), 323 (97), 279 (100), 91 (35); HRMS calcd for $C_{20}H_{32}N_2O_5^+$ 379.2233, found 379.2262.

***N*-Boc-*O*-benzyl-serine epoxide (7c)**: 97% yield. *Erythro:threo* 6.5:1. ^1H NMR *Erythro*: 7.335 (s, 5H), 5.070 (d, J=6.6 Hz, 1H), 4.549 (s, 2H), 3.718 (dd, J=9.5, 3.2 Hz, 1H), 3.590 (dd, J=9.4, 3.7 Hz, 1H), 3.52-3.40 (m, 1H), 3.089 (dt, J=7.2, 3.3 Hz, 1H), 2.870-2.765 (m, 2H), 1.440 (s, 9H); *Threo*: 3.52-3.40 (ob, 1H), 3.215 (dt, J=3.9, 2.6 Hz, 1H), 2.747 (dd, J=4.8, 4.1 Hz, 1H), 2.632 (dd, J=4.8, 2.8 Hz, 1H); ^{13}C NMR 155.33, 137.76, 128.43, 127.80, 127.65, 79.63, 73.46, 70.44, 52.53, 51.42, 47.09, 28.32; MS *m/z* 294 (5, MH^+), 238, (100), 194 (17); HRMS calcd for $C_{16}H_{24}NO_4^+$ 294.1705, found 294.1717.

***N*-Cbz-*O*-*t*-butyl-glutamate epoxide (7d)**: 99% yield from the corresponding bromomethyl ketone **5d**. *Erythro:threo* 9.4:1. 24% yield as a side product from the corresponding chloroketone **2d**. *Erythro:threo* 3:1. ^1H NMR *Erythro*: 7.342 (s, 5H), 5.086 (s, 2H), 5.12-5.00 (ob, 1H), 3.48 (m, 1H), 2.887 (dt, J=6.2, 3.2 Hz, 1H), 2.775 (d, J=2.8 Hz, 2H), 2.38 (dt, J=17, 7 Hz, 1H), 2.33 (dt, J=17, 7 Hz, 1H), 1.948 (ddt, J=14.2, 7.1, 4.6 1H), 1.87-1.65 (m, 1H), 1.422 (s, 9H); *Threo*: 3.48 (ob, 1H), 3.03 (m, 1H), 2.721 (t, J=4.3 Hz, 1H), 2.568 (dd, J=4.6, 2.8 Hz, 1H); ^{13}C NMR *Erythro*: 172.66, 156.04, 136.31, 128.49, 128.13, 128.03, 80.75, 66.86, 53.75, 52.81, 46.12, 31.78, 28.02, 26.37; *Threo*: 53.72, 44.11; MS (EI) *m/z* 336 (1, M^+), 280 (63), 236 (10), 91, (100); HRMS calcd for $C_{18}H_{25}NO_5^+$ 336.1811, found 336.1809.

***N*-Cbz-*O*-*t*-butyl-aspartate epoxide (7e)**: 92% yield from the corresponding bromomethyl ketone **5e**. *Erythro:threo* 6.1:1. ^1H NMR *Erythro*: 7.342 (s, 5H), 5.742 (d, J=8.8 Hz, 1H), 5.119 (d, J=12.3 Hz, 1H), 5.084 (d, J=12.2 Hz, 1H), 3.793 (ddt, J=8.8, 6.4, 5.3 Hz, 1H), 3.074 (ddd, J=6.4, 3.6, 2.8 Hz, 1H), 2.789 (dd, J=4.5, 3.6 Hz, 1H), 2.774 (dd, J=4.5, 2.8 Hz, 1H), 2.560 (d, J=5.3 Hz, 2H), 1.439 (s, 9H); *Threo*: 3.80-3.65 (ob, 1H), 3.153 (dt, J=4.2, 2.5 Hz, 1H), 2.743 (dd, J=4.4, 4.2 Hz, 1H), 2.604 (dd, J=4.6, 2.6 Hz, 1H); ^{13}C NMR 170.56, 155.78, 136.31, 128.49, 128.14, 128.06, 81.59, 66.88, 52.69, 49.83, 46.68, 36.55, 27.99; MS *m/z* 356 (7, MH^++34), 322 (2, MH^+), 266 (41), 265 (9), 222 (6), 158 (6), 92 (7), 91, (100); HRMS calcd for $C_{17}H_{24}NO_5^+$ 322.1654, found 322.1661.

Removal of *N*-Boc and *O*-*t*-Bu protecting groups. Chlorohydrins **3** or bromomethyl ketones **5** (1 mmol) were dissolved in 2 mL of CH_2Cl_2. TFA (2 mL) was added and the solution was stirred at rt. The reaction was followed by TLC (ether:hexane 1:1) and was typically completed within 1 h. The solvents were evaporated to dryness and the products (**4** or **6**, respectively) were transferred to the next reaction without further purification.

N_α-Cbz-lysine chlorohydrin (4b): 100% yield. ^{1}H NMR (in acetone-d_6) 7.343 (s, 5H), 5.080 (s, 2H), 3.85-3.65 (m, 2H), 3.85-3.65 (ob), 3.690 (dd, J=11.4, 3.8 Hz, 1H), 3.565 (dd, J=11.2, 6.7 Hz, 1H), 3.090 (bt, J=7.0 Hz), 1.94-1.34 (m, 6H); ^{13}C NMR 157.37, 138.12, 129.11, 128.52, 128.31, 74.33, 66.66, 53.97, 47.89, 40.37, 31-28 (ob), 27.51, 23.28. [TFA: 160.56, 117.12]; MS *m/z* 317, 315 (28, 100, MH^+), 279 (22), 115 (93), 91 (51), 84 (16); HRMS calcd for $C_{15}H_{24}N_2O_3{}^{35}Cl^+$ 315.1476, found 315.1470.

N-Cbz-glutamate chlorohydrin (4d): 38% yield after flash chromatography (CH_2Cl_2→EtOAc→MeOH). ^{1}H NMR (in D_2O) 7.426 (s, 5H), 5.118 (s, 2H), 3.90-3.47 (m, 4H), 2.33-2.12 (m, 2H), 2.05-1.50 (m, 2H); ^{13}C NMR 182.86, 158.46, 136.97, 129.20, 128.77, 127.99, 73.36, 66.99, 53.67, 47.10, 34.15, 27.11; MS *m/z* 280 (10, MH^+-HCl), 236 (10), 173 (12), 172 (77), 146 (25), 128 (17), 110 (14), 91 (100); HRMS calcd for $C_{14}H_{18}NO_5^+$ 280.1185, found 280.1189.

N_α-Cbz-lysyl bromomethane (6b): 93% yield. ^{1}H NMR (in acetone-d_6) 7.381 (s, 5H), 5.101 (s, 2H), 4.446 (dd, J=9.1, 4.3 Hz, 1H), 4.413 (s, 2H), 3.801 (t, J=7.1 Hz, 2H), 3.153 (t, J=7.4 Hz), 2.00-1.68 (m, 4H), 1.65-1.48 (m, 2H); ^{13}C NMR 201.31, 157.24, 138.00, 129.22, 128.71, 128.60, 67.04, 59.11, 47.81, 40.17, 34.39, 27.60, 23.52; MS (CI-NH_3) *m/z* 359, 357 (9, 8, MH^+), 277 (44), 169 (100).

N-Cbz-glutamyl bromomethane (6d): 99% yield. ^{1}H NMR (in acetone-d_6) 10.728 (s, 1H), 7.350 (s, 5H), 6.918 (d, J=7.7 Hz, 1H), 5.082 (s, 2H), 4.544 (ddd, J=9.4, 8.0, 4.6 Hz, 1H), 4.391 (s, 2H), 2.465 (t, J=7.1 Hz, 2H), 2.232 (dtd, J=15.2, 7.0, 4.5 Hz, 1H), 1.917 (ddt, J=14.2, 9.4, 6.9 Hz, 1H); ^{13}C NMR 200.99, 174.27, 157.22, 137.61, 129.17, 128.70, 128.60, 67.17, 58.47, 33.99, 30.20, 26.37; MS *m/z* 360, 358 (3, 3, MH^+), 324 (20), 280 (17), 278 (22), 236 (15), 234 (60), 190 (17), 117 (19), 100 (18), 99 (41), 91 (100); HRMS calcd for $C_{14}H_{17}NO_5{}^{79}Br^+$ 358.0290, found 358.0200; calcd for $C_{14}H_{17}NO_5{}^{81}Br^+$ 360.0270, found 360.0245.

N-Cbz-aspartyl bromomethane (6e): 94% yield. ^{1}H NMR (in acetone-d_6) 7.338 (s, 5H), 7.000 (d, J=8.0 Hz, 1H), 5.118 (s, 2H), 4.770 (m, 1H), 4.448 (bs, 2H), 2.973 (dd, J=16.9, 5.8 Hz, 1H), 2.870 (dd, J=17.0, 6.0 Hz, 1H); ^{13}C NMR 199.93, 172.25, 157.04, 137.84, 129.22, 128.73, 128.66, 67.29, 55.65, 35.89, 34.43; MS *m/z* 346, 344 (2, 2, MH^+), 264 (8), 92 (13), 91 (100); HRMS calcd for $C_{13}H_{15}NO_5{}^{79}Br^+$ 344.0134, found 344.0036.

N_α-Cbz-lysine epoxide 8b was prepared in 64% yield from the corresponding bromomethyl ketone **6b** and in 97% yield from the corresponding chlorohydrin **4b** (only the *erythro* isomer was observed by ^{1}H and ^{13}C NMR). The synthetic procedures were similar to those employed in the synthesis of the doubly protected lysine epoxide **7b**. ^{1}H NMR (CD_3OD) 7.327 (s, 5H), 5.057 (s, 2H), 3.404 (ddd, J=9.2, 6.1, 4.6 Hz, 1H), 2.864 (ddd, J=6.3, 3.8, 2.8 Hz, 1H), 2.67 (m, 2H), 2.600 (t, J=6.6 Hz, 2H), 1.78-1.20 (m, 6H); ^{13}C NMR 158.65, 129.41, 128.94, 128.72, 67.50, 54.93, 53.87, 46.02, 42.30, 33.42, 32.62, 24.11; MS *m/z* 279 (15, MH^+), 91 (100); HRMS calcd for $C_{15}H_{23}N_2O_3^+$ 279.1709, found 279.1674.

N-Boc-serine epoxide 8c was prepared from *N*-Boc-*O*-Bn-serine epoxide **7c** by catalytic transfer hydrogenation according to a published procedure[21] in 14% yield, along with an unidentified side product. *Erythro:threo* 6.5:1. ^{1}H NMR (in acetone-d_6) *Erythro*: 5.88 (bs, 1H), 3.80-3.50 (m, 2H), 3.35 (m, 1H), 2.976

(dt, J=6.6, 3.3 Hz, 1H), 2.692 (d, J=3.2 Hz, 2H), 1.402 (s, 9H); *Threo*: 3.60-3.50 (m, 1H), 3.120 (td, J=3.9, 2.8 Hz, 1H), 2.692 (dd, J=5.2, 3.9 Hz, 1H), 2.554 (dd, J=5.1, 2.7 Hz, 1H); ^{13}C NMR 155.84, 80.00, 62.25, 53.59, 52.07, 46.11, 28.36; MS *m/z* 204 (11, MH^+), 148 (100), 104 (21); HRMS calcd for $C_9H_{18}NO_4^+$ 204.1236, found 204.1198.

***N*-Boc-aspartate bromomethyl lactone (9)**: MS *m/z* 238, 240 (99, 100, MH^+-CH_2=$C(CH_3)_2$), 158 (29); HRMS calcd for $C_6H_9NO_4{}^{79}Br^+$ 237.9715, found 237.9688.

***N*-Boc-*O*-ethyl-aspartate epoxide (10)**: 11% yield, after flash chromatography (CH_2Cl_2). *Erythro:threo* 9.3:1. ^{1}H NMR *Erythro*: 5.374 (bs, 1H), 4.169 (q, J=7.1 Hz, 2H), 3.85-3.67 (m, 1H), 3.084 (ddd, J=6.5, 3.8, 2.6 Hz, 1H), 2.806 (dd, J=4.8, 4.0 Hz, 1H), 2.741 (dd, J=4.9, 2.7 Hz, 1H), 2.627 (d, J=5.3 Hz, 2H), 1.442 (s, 9H), 1.275 (t, J=7.2 Hz, 3H); *Threo*: 3.134 (ddd, J=6.8, 4.0, 2.2 Hz, 1H), 2.78-2.58 (ob, 2H); ^{13}C NMR *Erythro*: 171.35, 155.14, 79.82, 66.81, 52.86, 49.34, 46.80, 35.72, 28.36, 14.14; *Threo*: 51.20, 44.51; MS *m/z* 260 (13, MH^+), 204 (100), 160 (51); HRMS calcd for $C_{12}H_{22}NO_5^+$ 260.1498, found 260.1482.

***N*-Cbz-glutamate bromohydrin (11)**: 68% yield. *Erythro:threo* 2.9:1. ^{1}H NMR (in acetone-d_6) *Erythro*: 7.361 (m, 5H), 6.324 (d, J=9.2 Hz, 1H), 5.077 (s, 2H), 3.851 (td, J=7.0, 3.4 Hz, 1H), 3.748 (tdd, J=9.5, 6.3, 3.3 Hz, 1H), 3.620 (dd, J=10.7, 3.8 Hz, 1H), 3.454 (dd, J=10.5, 7.5 Hz, 1H), 2.419 (dt, J=16.6, 5.6 Hz, 1H), 2.363 (dt, J=16.5, 7.0 Hz, 1H), 2.1 (part. ob., 1H), 1.782 (dddd, J=13.6, 10.6, 8.7, 6.3 Hz, 1H); *Threo*: 6.072 (d, J=9.2 Hz, 1H), 3.96-3.66 (ob, 2H), 3.533 (dd, J=10.4, 4.8 Hz, 1H), 3.366 (dd, J=10.3, 7.4 Hz, 1H), 1.906 (q, J=7.4 Hz, 2H); ^{13}C NMR *Erythro*: 174.80, 157.15, 138.30, 129.17, 128.56, 128.50, 74.50, 66.68, 54.99, 37.31, 30.18, 25.94; *Threo*: 73.98, 54.12, 36.44; MS *m/z* 362, 360 (1, 1, MH^+), 91 (100).

***N*-Cbz-glutamate chloromethyl lactone (12)**: MS *m/z* 300, 298 (12, 18, MH^+), 192, 190 (4, 11), 91 (100); HRMS calcd for $C_{14}H_{17}NO_4{}^{35}Cl^+$ 298.0846 found 298.0843.

Enzymatic Assays. Cathepsin B and papain were activated and assayed as previously described.[7c] Trypsin was assayed according to a published procedure.[7a]

Inactivation Studies were carried out as previously described.[7c]

Acknowledgment. This research was supported by The Basic Research Foundation administered by The Israeli Academy of Sciences and Humanities.

REFERENCES AND NOTES

1. (a) Bartók, M.; Láng, K. L. in *The Chemistry of Ethers, Crown Ethers, Hydroxyl Groups and their Sulfur Analogs*. Part 2, Supp. E; Patai, S. Ed.; John Wiley and Sons, Inc.: New York, 1980; pp 655-659. (b) Rao, A. S.; Paknikar, S. K.; Kirtane, J. G. *Tetrahedron* **1983**, *39*, 2323-2367. (c) Parker, R. E.; Isaacs, N. S. *Chem. Rev.* **1959**, *59*, 737-799.
2. George, P.; Bock, C. W.; Glusker, J. P. *J. Phys. Chem.* **1992**, *96*, 3702-3708.
3. (a) Thompson, W. J.; Fitzgerald, P. M. D.; Holloway, M. K.; Emini, E. A.; Darke, P. L.; McKeever, B. M.; Schleif, W. A.; Quintero, J. C.; Zugay, J. A.; Tucker, T. J.; Schwering, J. E.; Homnick, C. F.; Nunberg, J.; Springer, J. P.; Huff, J. R. *J. Med. Chem.* **1992**, *35*, 1685-1701. (b) Luly, J. R.; Bolis,

G.; BaMaung, N.; Soderquist, J.; Dellaria, J. F.; Stein, H.; Cohen, J.; Perun, T. J.; Greer, J.; Plattner, J. J. *J. Med. Chem.* **1988**, *31*, 532-539. (c) Dellaria, J. F.; Maki, R. G.; Bopp, B. A.; Cohen, J.; Kleinert, H. D.; Luly, J. R.; Merits, I.; Plattner, J. J.; Stein, H. H. *J. Med. Chem.* **1987**, *30*, 2137-2144. (d) Luly, J. R.; Yi, N.; Soderquist, J.; Stein, H.; Cohen, J.; Perun, T. J.; Plattner, J. J. *J. Med. Chem.* **1987**, *30*, 1609-1616. (e) Luly, J. R.; Plattner, J. J.; Stein, H.; Yi, N.; Soderquist, J.; Marcotte, P. A.; Kleinert, H. D.; Perun, T. J. *Biochem. Biophys. Res. Commun.* **1987**, *143*, 44-51. (f) Askin, D.; Wallace, M. A.; Vacca, J. P.; Reamer, R. A.; Volante, R. P.; Shinkai, I. *J. Org. Chem.* **1992**, *57*, 2771-2773. (g) Rosenberg, S. H.; Plattner, J. J.; Woods, K. W.; Stein, H. H.; Marcotte, P. A.; Cohen, J.; Perun, T. J. *J. Med. Chem.* **1987**, *30*, 1224-1228. (h) Rich, D. H.; Sun, C.-Q.; Vara Prasad, J. V. N.; Pathiasseril, A.; Toth, M. V.; Marshall, G. R.; Clare, M.; Mueller, R. A.; Houseman, K. *J. Med. Chem.* **1991**, *34*, 1222-1225.

4. (a) Ohfune, Y.; Kurokawa, N. *Tetrahedron Lett.* **1984**, *25*, 1587-1590. (b) Kurokawa, N.; Ohfune, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6041-6043. (c) Shaw, K. J.; Luly, J. R.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 4515-4523. (d) Jones, R. J.; Rapoport, H. *J. Org. Chem.* **1990**, *55*, 1144-1146.
5. (a) Meffre, P.; Vo-Quang, L.; Vo-Quang, Y.; Le Goffic, F. *Tetrahedron Lett.* **1990**, *31*, 2291-2294. (b) Tashiro, T.; Fushiya, S.; Nozoe, S. *Chem. Pharm. Bull.* **1988**, *36*, 893-901.
6. Baker, R.; Leeson, P. D.; Williams, B. J. *Chem. Abstr.* **1992**, *116*, 214339e.
7. (a) Giordano, C.; Gallina, C.; Consalvi, V.; Scandurra, R. *Eur. J. Med. Chem.* **1990**, *25*, 479-487. (b) Albeck, A.; Persky, R.; Kliper, S. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1767-1772. (c) Albeck, A.; Fluss, S.; Persky, R. *J. Am. Chem. Soc.* **1996**, 118, 3591-3596.
8. (a) Bühlmayer, P.; Caselli, A.; Fuhrer, W.; Göschke, R.; Rasetti, V.; Rüeger, H.; Stanton, J. L.; Criscione, L.; Wood, J. M. *J. Med. Chem.* **1988**, *31*, 1839-1846. (b) Ashton, W. T.; Cantone, C. L.; Meurer, L. C.; Tolman, R. L.; Greenlee, W. J.; Patchett, A. A.; Lynch, R. J.; Schorn, T. W.; Strouse, J. F.; Siegl, P. K. S. *J. Med. Chem.* **1992**, *35*, 2103-2112.
9. (a) Luly, J. R.; Dellaria, J. F.; Plattner, J. J.; Soderquist, J. L.; Yi, N. *J. Org. Chem.* **1987**, *52*, 1487-1492. (b) Romeo, S.; Rich, D. H. *Tetrahedron Lett.* **1993**, *34*, 7187-7190. (c) Albeck, A.; Persky, R. *J. Org. Chem.* **1994**, *59*, 653-657.
10. Barluenga, J.; Baragaña, B.; Concellón, J. M. *J. Org. Chem.* **1995**, *60*, 6696-6699.
11. (a) Albeck, A.; Persky, R. *Tetrahedron* **1994**, *50*, 6333-6346. (b) Rotella, D. P. *Tetrahedron Lett.* **1995**, *36*, 5453-5456.
12. Green, B. E.; Chen, X.; Norbeck, D. W.; Kempf, D. J. *Synlett* **1995**, 613-614.
13. Reetz, M. T.; Binder, J. *Tetrahedron Lett.* **1989**, *30*, 5425-5428.
14. Pégorier, L.; Petit, Y.; Larchevêque, M. *J. Chem. Soc., Chem. Commun.* **1994**, 633-634.
15. Two single examples of stereoselective syntheses of *threo* α-amino epoxides bearing functionalized side chains are in ref. 4a and 4c.
16. (a) Kraut, J. *Annu. Rev. Biochem.* **1977**, *46*, 331-358. (b) Steitz, T.; Shulman, R. *Annu. Rev. Biophys. Bioeng.* **1982**, *11*, 419-444.
17. (a) Rich, D. H. in *Proteinase Inhibitors* (Research Monographs in Cell and Tissue Physiology, Vol. 12); Barrett, A. J.; Salvesen, G. Eds.; Elsevier: Amsterdam, 1986; pp 153-178. (b) Lowe, G. *Tetrahedron* **1976**, *32*, 291-302.
18. Thornberry, N. A.; Molineaux, S. M. *Protein Science* **1995**, *4*, 3-12.

19. Libby, R.T.; Cosman, D.; Cooney, M. K.; Merriam, J. E.; March, C. J.; Hopp, T. P. *Biochemistry* **1988**, *27*, 6262-6268.
20. A dipeptidyl epoxide based on *O*-benzyl threonine, bearing an ether functionality in its side chain, was recently reported by us. See ref. 7c.
21. Felix, A. M.; Heimer, E. P.; Lambros, T. J.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1978**, *3*, 4194-4196.
22. Pretsch, E.; Clerc, T.; Seibl, J.; Simon, W. *Tables of Spectral Data for Structure Determination of Organic Compounds*, 2nd ed.; Springer-Verlag: New York, **1989**; p. H65.
23. The P and P' for substrates and S and S' for enzyme subsites terminology is used. See Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157-162.
24. Kitz, R.; Wilson, I. B. *J. Biol. Chem.* **1962**, *237*, 3245-3249.
25. Powers, J. C. in *Chemistry and Biology of Amino Acids, Peptides and Proteins*, Vol. 4; Weinstein, B. Ed.; Marcel Dekker: New York, 1977; pp 66-178.
26. Ueda, T.; Kam, C.-M.; Powers, J. C. *Biochem. J.* **1990**, *265*, 539-545.
27. Aoyagi, T.; Miyata, S.; Nanbo, M.; Kojima, F.; Matsuzaki, M.; Ishizuka, M.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1969**, *22*, 558-568.
28. (a) Howard, A. E.; Kollman, P. A. *J. Am. Chem. Soc.* **1988**, *110*, 7195-7200. (b) Arad, D.; Langridge, R.; Kollman, P. A. *J. Am. Chem. Soc.* **1990**, *112*, 491-502.
29. Krebs, K. G.; Heusser, D.; Wimmer, H. in *Thin Layer Chromatography* 2nd ed.; Stahl, E. Ed.; Springer Verlag: New York, 1969; p. 862.
30. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923-2925.

(*Received in UK* 22 *January* 1997; *revised* 14 *February* 1997; *accepted* 20 *February* 1997)