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Synthesis of Dipeptide-Bound Epoxides and α,β-Unsaturated Amides as Potential Irreversible Transglutaminase Inhibitors

Pierre de Macédo, Claudio Marrano and Jeffrey W. Keillor*

Département de chimie, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montréal, Québec, Canada H3C 3J7

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Abstract—Herein we report the synthesis of 24 novel peptides as potential irreversible inactivators of transglutaminase (TGase). These peptides were designed to resemble Cbz-L-Gln-Gly, known to be a good TGase substrate, and to include either α,β-unsaturated amide groups or the corresponding epoxide groups. The side chain length of the amino acid residue bearing the inhibitor group was also varied in order to permit investigation of this effect.

Introduction

Transglutaminases (TGases, EC 2.3.2.13) constitute a class of calcium-dependent acyltransferases that catalyze the formation of an amide bound between the γ-carboxamide groups of peptide-bound glutamine residues and various primary amines. These enzymes are widely distributed in mammalian tissues, plasma and epidermis. Tissue TGases are involved in diverse biological functions such as endocytosis,[1,2] apoptosis[3] and cell growth regulation.[4] Plasma-soluble TGase (Factor XIIIa) catalyses the formation of fibrin cross-links, stabilizing blood clots during their formation.[5–7] Epidermal TGases are responsible for the formation of the cornified envelope of epidermal keratinocytes.[8–11]

High TGase activities have also been implicated in a number of disease states, including acne,[12,13] cataracts,[14] immunologic diseases,[15] psoriasis,[16–18] and Alzheimer's disease.[19–25] Inhibitors may be useful in the treatment of TGase dysfunction. The TGase mechanism involves the transfer of an acyl group from a substrate to an active site cysteine thiol residue of the enzyme.[26] TGases are therefore inactivated by α,β-unsaturated amides (Michael acceptors) and epoxide derivatives,[27] as are many enzymes with an active site thiol group, through the nucleophilic attack of the cysteine thiol on the inhibitor resulting in the formation of a covalent bond that is resistant to subsequent hydrolysis or aminolysis reactions. During the past decades, TGase has been shown to be irreversibly inactivated by a number of different compounds, including sulfonamides,[28,29] iodoacetates,[30–32] isocyanates,[33] thioureas,[34] acivicin derivatives,[35,36] sulfonium methyl ketones,[37] thioacetonyl heterocycles,[38] and electrophilic glutamine analogues.[39] However, their lack of specificity limits their therapeutic utility. Herein, we report the synthesis of potential inhibitors designed to have high TGase affinity.

Results and Discussion

The basic structure of the inhibitors shown herein is based on that of carbobenzyloxy-L-glutaminylglycine (Cbz-Gln-Gly), a commonly used dipeptide TGase substrate.[26,40,41] In the enzymatic mechanism, the acylation step consists of the attack of the active site cysteine thiol residue on the carbonyl of the γ-carboxamide function of the substrate glutamine side chain. We therefore chose to place reactive functional groups at the end of the peptide side chain. Furthermore, we designed analogues with different chain lengths in order to study the effect of this variation.

The starting points of our parallel syntheses were the corresponding side chain primary amine derivatives. Products **3a** and **4a** are commercially available, whereas products **1a**–**2a** were obtained after the rearrangement of their corresponding amides N_α-carbobenzyloxy-L-asparagine and N_α-carbobenzyloxy-L-glutamine respectively (Scheme 1).[42] This reaction was carried out in the presence of bis(trifluoroacetoxy)phenyliodine and the desired products were obtained after precipitation with yields around 80%.

Compounds **1b**–**4b** were prepared by allowing acryloyl chloride to react with compounds **1a**–**4a** (where the

*Corresponding author. Tel.: +1-514-343-6219; fax: +1-514-343-7586; e-mail: keillorj@chimie.unmontreal.ca

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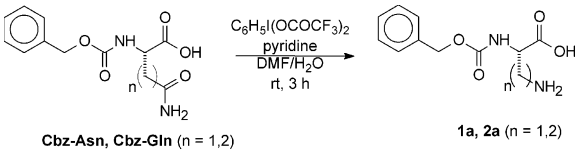
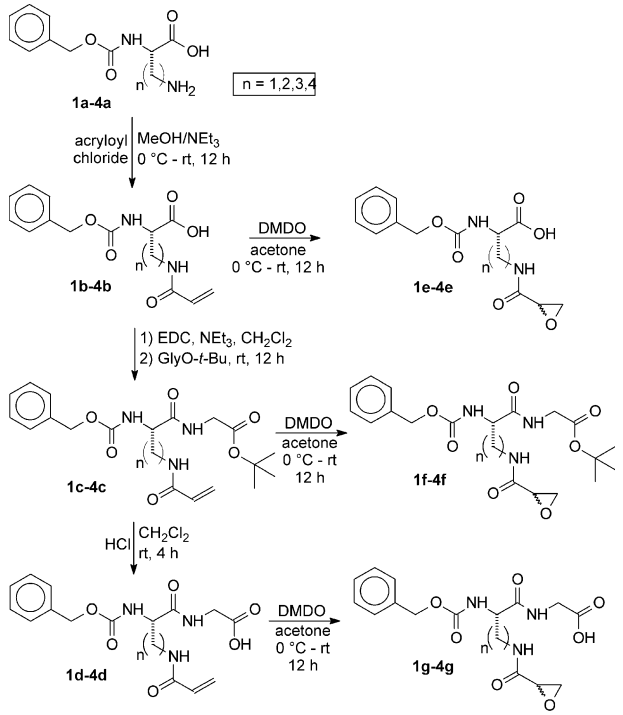
compound number corresponds to the number of methylene units of the side chain) in the presence of triethylamine in methanol (Scheme 2). The isolated yields varied from 73–90% according to the length of the side chain, since the less polar molecules were more easily isolated during the work up conditions.

The following step consisted of the coupling of *tert*-butyl glycine ester with products **1b–4b**, previously activated by 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide (EDC) in the presence of triethylamine in dichloromethane. Compounds **1c–4c** were thus obtained with yields between 70 and 93%, the less polar derivatives being once again more easily isolated. The *tert*-butyl ester groups were hydrolyzed by bubbling hydrogen chloride through dichloromethane solutions. In this way, products **1d–4d** were obtained in quantitative yields. Compounds **1b–4d** bear an α,β-unsaturated amide and are thus potential irreversible (Michael acceptor) inhibitors of cysteine-activity dependent enzymes.

These compounds also serve as the synthetic precursors of the corresponding epoxide analogues, which also have the potential of forming irreversible adduct products with active site thiol residues. A novel method using dimethyldioxirane (DMDO),[43–45] which is the peroxide of acetone, was used to transform the



Scheme 1.



Scheme 2.

compounds bearing a Michael acceptor function (**1b–4d**) into the corresponding epoxides **1e–4g**. DMDO was first described by Adam et al.[43] and has been employed as a reagent that is particularly useful for the epoxidation of conjugated double bonds,[44] as a preferred alternative to m-CPBA, magnesium peroxyphthalate, hydrogen peroxide in basic conditions and hydrogen peroxide with tungsten tetroxide.[45] Since the epoxidation reactions are quantitative, this method is advantageous because pure product is obtained the after evaporation of excess DMDO and acetone. Using this method, compounds **1e–4g** were obtained in essentially quantitative yield, since no further purification was required after the transformation of starting materials **1b--4d**. Like classical epoxidation methods, this procedure resulted in the racemic formation of the epoxide group. Due to the presence of an additional stereocenter on the Cbz-Gln-Gly scaffold, each epoxide dipeptide was thus obtained as a mixture of two diastereoisomers.

Conclusion

Efficient synthetic routes have been developed for the synthesis of 24 new potential irreversible inhibitors of TGases. All of these compounds are analogues of Cbz-Gln-Gly, a commonly used substrate for TGases. Based on this similarity of structure and the known irreversible inhibition efficacy of Michael acceptor and epoxide functional groups, we expect to obtain promising enzymatic inhibition results. The results of our studies of the inhibitory activity of these compounds, using a TGase activity assay recently developed in our laboratories,[46] will be reported shortly.[47]

Experimental

Materials and methods

^1H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer. Solvents are indicated in the text and the chemical shifts are reported in ppm with internal reference to TMS. Mass spectra (MS) were recorded on a Micromass 1212 spectrometer. Infrared (IR) spectra were recorded in the range 4000–600 cm^{-1} using a Perkin-Elmer 298 infrared spectrometer. Spectra of liquids were taken as films of $CHCl_3$ solutions between NaCl plates and spectra of solids with KBr disks. Melting points (mp) were determined with a capillary tube Thomas Hoover melting point apparatus and are reported as uncorrected values. Flash chromatography was carried out on silica gel (200–430 mesh) obtained from Silicycle. The starting compounds were obtained from Sigma-Aldrich.

General amide rearrangement procedure A: synthesis of compounds 1a–2a. To a solution of 6.45 g of bis(trifluoroacetoxy)phenyliodine (15 mmol) in dimethylformamide/water (60 mL; 1:1 v/v) were added at room temperature 2.66 g of N_α-carbobenzyloxy-L-asparagine or N_α-carbobenzyloxy-L-glutamine (10 mmol). After 15 min, 1.6 mL of pyridine (20 mmol) was added and

stirring was continued for 3 h. The solvents were removed under reduced pressure and the residue was dissolved in water (100 mL). The solution was washed with diethyl ether and the aqueous layer was evaporated under reduced pressure. Crude compounds **1a** or **2a** were dissolved in ethanol and precipitated with ether. The precipitate was filtered and washed with ether to give pure product.

General acryloyl amidation procedure B: synthesis of compounds 1b–4b. In 300 mL of methanol 10 mmol of the corresponding amino derivatives **1a**–**4a** was dissolved under nitrogen. The solution was cooled to 0 °C. After the addition of 14 mL of triethylamine (100 mmol), 34.8 mL of acryloyl chloride (60 mmol) was added dropwise. The mixture was allowed to warm to room temperature overnight with stirring. The reaction mixture was then evaporated under reduced pressure and the residue was dissolved in water (100 mL). The pH was adjusted around to 1.5 with 1 M HCl, and the product was extracted with ethyl acetate (3×100 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure to give pure product.

General peptide coupling procedure C: synthesis of compounds 1c–4c. Compounds **1b**–**4b** (6 mmol) were activated by reaction with 1.15 g of EDC hydrochloride (6 mmol) and 0.92 mL of triethylamine (6.6 mmol) in dichloromethane (50 mL). After 15 min, 1.00 g of glycine *tert*-butyl ester hydrochloride (6 mmol) was added and the mixture was stirred overnight at room temperature. The mixture was then washed with water (3×100 mL) and the organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography (ethyl acetate eluant) to give pure product.

General *tert*-butyl ester deprotection procedure D: synthesis of compounds 1d–4d. Compounds **1c**–**4c** (3 mmol) were dissolved in dichloromethane (50 mL). Gaseous hydrochloric acid was bubbled through the solution for 4 h at room temperature. The solvent was removed under reduced pressure to give pure product.

General epoxidation procedure E: synthesis of compounds 1e–4e, 1f–4f, 1g–4g. DMDO was prepared by distillation under reduced pressure from a mixture of 58 g of $NaHCO_3$ (690 mmol), 120 g of OXONE® (potassium peroxymonosulfate, 178 mmol) in water (250 mL) and acetone (192 mL) using a dry ice trap. A large excess of a freshly prepared 2 M solution of DMDO in acetone was then added to compounds **1b**–**4b**, **1c**–**4c**, and **1d**–**4d** (1 mmol) in order to complete the epoxidation. The mixture was stirred for 5 h at 0 °C and then allowed to warm to room temperature overnight with stirring. The solvent and any excess DMDO were subsequently removed under reduced pressure to give pure product.

Spectral data

N_α-Carbobenzyloxy-L-2,3-diaminopropionic acid (1a). This compound was prepared using general procedure A to give the product as a white powder (80% yield, mp 212 °C). ^{1}H NMR (D_2O, 400 MHz) δ 7.21–7.13 (m, 5H), 4.38 (s, 2H), 3.98 (t, 1H, J = 10.0 Hz), 3.55 (d, 2H, J = 10.0 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 178.59, 158.76, 137.14, 129.53, 129.11, 128.51, 67.71, 59.72, 43.58. IR ν_{max} (cm^{-1}) 3130 (OH); 3050 (NH_2); 1751, 1690 (C=O). MS (FAB+) 239.1 (MH+).

N_α-Carbobenzyloxy-L-2,4-diaminobutyric acid (2a). This compound was prepared using general procedure A to give the product as a white powder (81% yield, p 191 °C). ^{1}H NMR (D_2O, 400 MHz) δ 7.25–7.14 (m, 5H), 4.40 (s, 2H), 3.94 (t, 1H, J = 9.9 Hz), 3.59 (t, 2H, J = 5.0 Hz), 2.11 (td, 2h, J = 9.8 Hz, J = 5.0 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 178.52, 158.57, 137.09, 129.51, 129.12, 128.47, 67.77, 54.68, 37.48, 30.50. IR ν_{max} (cm^{-1}) 3115 (OH); 3010 (NH_2); 1748, 1692 (C=O). MS (FAB+) 253.1 (MH+)

N_α-Carbobenzyloxy-L-2-amino-3-acryloylaminopropionic acid (1b). This compound was prepared using general procedure B to give the product as a light-yellow oil (73% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.93 (s, 1H), 7.30–7.26 (m, 5H), 7.03 (s, 1H), 6.21 (t, 1H, J = 8.1 Hz), 6.07 (d, 2H, J = 8.2 Hz), 5.51 (s, 1H), 5.03 (s, 2H), 4.27 (t, 1H, J = 5.3 Hz), 3.27 (d, 2H, J = 5.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.80, 167.65, 156.58, 136.07, 130.10, 128.49, 128.15, 127.98, 127.59, 67.09, 54.61, 41.32. IR ν_{max} (cm^{-1}) 3310 (OH); 1746, 1690, 1663 (C=O). MS (FAB+) 293.1 (MH+).

N_α-Carbobenzyloxy-L-2-amino-4-acryloylaminobutyric acid (2b). This compound was prepared using general procedure B to give the product as a light orange oil (80% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.94 (s, 1H), 7.27–7.23 (m, 5H), 7.02 (s, 1H), 6.19 (t, 1H, J = 7.9 Hz), 6.05 (d, 2H, J = 7.9 Hz), 5.48 (s, 1H), 5.05 (s, 2H), 4.27 (t, 1H, J = 4.8 Hz), 3.23 (t, 2H, J = 4.7 Hz), 2.11 (td, 2H, J = 4.8 Hz, J = 4.8 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.18, 167.01, 156.70, 136.10, 130.43, 128.44, 128.08, 127.84, 126.97, 66.97, 51.80, 36.07, 31.75. IR ν_{max} (cm^{-1}) 3314 (OH); 1743, 1694, 1669 (C=O). MS (FAB+) 307.1 (MH+).

N_α-Carbobenzyloxy-L-2-amino-5-acryloylaminopentanoic acid (3b). This compound was prepared using general procedure B to give the product as a white powder (83% yield, mp 60 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.95 (s, 1H), 7.30–7.26 (m, 5H), 7.01 (s, 1H), 6.17 (t, 1H, J = 8.1 Hz), 6.01 (d, 2H, J = 7.9 Hz), 5.50 (s, 1H), 5.02 (s, 2H), 4.25 (t, 1H, J = 4.8 Hz), 3.20 (t, 2H, J = 4.7 Hz), 1.75–1.35 (m, 4H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.08, 164.22, 153.94, 133.66, 128.05, 125.93, 125.57, 125.37, 124.25, 64.37, 51.03, 36.59, 26.99, 22.66. IR ν_{max} (cm^{-1}) 3310 (OH); 1744, 1691, 1668 (C=O). MS (FAB+) 321.1 (MH+).

N_α-Carbobenzyloxy-L-2-amino-6-acryloylaminohexanoic acid (4b). This compound was prepared using general procedure B to give the product as an orange powder (90% yield, mp 65 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.93 (s, 1H), 7.30–7.27 (m, 5H), 7.04 (s, 1H), 6.17 (t, 1H, J = 7.9 Hz), 6.02 (d, 2H, J = 7.9 Hz), 5.50 (s, 1H), 5.04 (s,

2H), 4.27 (t, 1H, $J=4.6$ Hz), 3.21 (t, 2H, $J=4.5$ Hz), 1.81–1.26 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.68, 166.51, 156.34, 136.08, 130.51, 128.34, 127.97, 127.77, 126.56, 66.77, 53.57, 39.11, 31.58, 28.44, 22.35. IR ν_{max} (cm^{-1}): 3305 (OH); 1740, 1691, 1665 (C=O). MS (FAB+) 335.1 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-3-acryloylamino)propionylglycine *tert*-butyl ester (1c). This compound was prepared using general procedure C to give the product as a soft white powder (70% yield, mp 70 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.22 (m, 5H), 6.82 (s, 1H), 6.21 (t, 1H, $J=8.2$ Hz), 6.09 (d, 2H, $J=8.1$ Hz), 5.49 (s, 1H), 5.47 (s, 1H), 5.01 (s, 2H), 4.19 (t, 1H, $J=5.7$ Hz), 3.91 (s, 2H), 3.33 (d, 2H, $J=5.9$ Hz), 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.96, 168.80, 166.76, 156.35, 136.21, 130.75, 128.23, 128.06, 127.67, 126.18, 81.71, 66.57, 55.36, 41.26, 41.29, 27.77. IR ν_{max} (cm^{-1}) 1753, 1739, 1657, 1648 (C=O). MS (FAB+) 406.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-4-acryloylamino)butyrylglycine *tert*-butyl ester (2c). This compound was prepared using general procedure C to give the product as a white powder (80% yield, mp 120 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28–7.21 (m, 5H), 6.80 (s, 1H), 6.18 (t, 1H, $J=8.2$ Hz), 6.09 (d, 2H, $J=8.0$ Hz), 5.48 (s, 1H), 5.45 (s, 1H), 5.00 (s, 2H), 4.18 (t, 1H, $J=5.6$ Hz), 3.89 (s, 2H), 3.30 (t, 2H, $J=6.1$ Hz), 1.93 (td, 2H, $J=5.8$ Hz, $J=5.7$ Hz), 1.45 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.14, 168.74, 166.47, 156.29, 136.23, 130.86, 128.38, 127.97, 126.19, 81.85, 66.74, 53.45, 41.94, 35.86, 27.91. IR ν_{max} (cm^{-1}) 1759, 1746, 1664, 1651 (C=O). MS (FAB+) 420.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-5-acryloylamino)pentanoylglycine *tert*-butyl ester (3c). This compound was prepared using general procedure C to give the product as a yellow powder (85% yield, mp 84 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.29–7.18 (m, 5H), 6.77 (s, 1H), 6.17 (t, 1H, $J=8.0$ Hz), 6.06 (d, 2H, $J=8.1$ Hz), 5.47 (s, 1H), 5.44 (s, 1H), 5.02 (s, 2H), 4.18 (t, 1H, $J=5.0$ Hz), 3.80 (s, 2H), 3.24 (t, 2H, $J=6.0$ Hz), 1.95–1.27 (m, 13H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.20, 166.23, 163.59, 153.84, 133.73, 128.55, 125.76, 125.33, 125.21, 123.19, 79.12, 64.03, 51.63, 39.27, 35.94, 27.41, 25.29, 22.81. IR ν_{max} (cm^{-1}) 1755, 1746, 1662, 1654 (C=O). MS (FAB+) 434.1 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-6-acryloylamino)hexanoylglycine *tert*-butyl ester (4c). This compound was prepared using general procedure C to give the product as a white powder (93% yield, mp 116 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.30–7.19 (m, 5H), 6.78 (s, 1H), 6.17 (t, 1H, $J=8.1$ Hz), 6.08 (d, 2H, $J=8.0$ Hz), 5.49 (s, 1H), 5.45 (s, 1H), 5.02 (s, 2H), 4.20 (t, 1H, $J=5.3$ Hz), 3.80 (s, 2H), 3.26 (t, 2H, $J=6.2$ Hz), 1.96–1.20 (m, 15H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.55, 168.67, 165.91, 156.34, 136.10, 130.93, 128.27, 127.88, 127.73, 125.78, 81.76, 66.62, 54.65, 41.76, 38.68, 31.82, 28.57, 27.80, 22.38. IR ν_{max} (cm^{-1}) 1752, 1745, 1660, 1652 (C=O). MS (FAB+) 448.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-3-acryloylamino)propionylglycine (1d). This compound was prepared using general procedure D to give the product as a soft white powder (100% yield, mp 115 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.35–7.28 (m, 5H), 6.17 (d, 2H, $J=8.0$ Hz), 5.55 (t, 1H, $J=8.1$ Hz), 5.08 (s, 2H), 4.21 (t, 1H, $J=6.5$ Hz), 3.72 (s, 2H), 3.21 (d, 2H, $J=6.6$ Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.10, 171.87, 168.90, 158.28, 137.98, 131.82, 129.64, 129.19, 128.99, 127.68, 67.96, 56.50, 42.13, 41.26. IR ν_{max} (cm^{-1}) 3318 (OH); 1745, 1689, 1658, 1649 (C=O). MS (FAB+) 350.1 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-4-acryloylamino)butyrylglycine (2d). This compound was prepared using general procedure D to give the product as a soft-white powder (100% yield, mp 124 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.34–7.29 (m, 5H), 6.19 (d, 2H, $J=8.3$ Hz), 5.60 (t, 1H, $J=8.3$ Hz), 5.04 (s, 2H), 4.18 (t, 1H, $J=6.7$ Hz), 3.72 (s, 2H), 3.20 (t, 2H, $J=6.5$ Hz), 2.18 (td, 2H, $J=6.4$ Hz, $J=6.6$ Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.00, 172.88, 168.60, 158.37, 137.99, 131.64, 129.62, 129.17, 128.96, 127.67, 67.93, 54.40, 42.03, 37.50, 32.86. IR ν_{max} (cm^{-1}) 3311 (OH); 1749, 1692, 1660, 1651 (C=O). MS (FAB+) 364.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-5-acryloylamino)pentanoylglycine (3d). This compound was prepared using general procedure D to give the product as a soft white powder (100% yield, mp 144 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.36–7.30 (m, 5H), 6.23 (d, 2H, $J=8.5$ Hz), 5.69 (t, 1H, $J=8.4$ Hz), 5.07 (s, 2H), 4.14 (t, 1H, $J=6.3$ Hz), 3.74 (s, 2H), 3.19 (t, 2H, $J=6.5$ Hz), 1.98–1.42 (m, 4H). ^{13}C NMR (CD_3OD, 100 MHz) δ 175.32, 171.67, 168.72, 158.30, 138.13, 131.18, 129.70, 129.23, 129.00, 128.45, 67.84, 56.10, 42.10, 40.55, 30.83, 26.55. IR ν_{max} (cm^{-1}) 3313 (OH); 1747, 1694, 1662, 1654 (C=O). MS (FAB+) 378.1 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-6-acryloylamino)hexanoylglycine (4d). This compound was prepared using general procedure D to give the product as a light green powder (100% yield, mp 116 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.36–7.30 (m, 5H), 6.22 (d, 2H, $J=8.6$ Hz), 5.64 (t, 1H, $J=8.4$ Hz), 5.09 (s, 2H), 4.15 (t, 1H, $J=6.5$ Hz), 3.72 (s, 2H), 3.26 (t, 2H, $J=6.5$ Hz), 1.90–1.40 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.59, 171.67, 168.68, 158.33, 138.11, 131.20, 129.68, 129.22, 128.96, 128.33, 67.81, 56.51, 42.06, 40.74, 32.96, 29.71, 24.17. IR ν_{max} (cm^{-1}) 3310 (OH); 1745, 1690, 1660, 1650 (C=O). MS (FAB+) 392.4 (MH+).

N_α-Carbobenzyloxy-L-2-amino-3-(oxiranecarbonylamino)-propionic acid (1e). This compound was prepared using general procedure E to give the product as a light-yellow oil (100% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.33–7.29 (m, 5H), 5.12 (s, 2H), 4.21 (t, 1H, $J=6.9$ Hz), 3.33 (t, 1H, $J=4.7$ Hz), 3.25 (d, 2H, $J=7.1$ Hz), 2.80 (d, 2H, $J=4.6$ Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.43, 172.08, 158.45, 138.04, 129.57, 129.12, 128.94, 67.83, 55.15, 49.99, 47.64, 41.25. IR ν_{max} (cm^{-1}) 3340 (OH); 1750, 1700, 1686 (C=O). MS (FAB+) 309.1 (MH+).

N_α-Carbobenzyloxy-L-2-amino-4-(oxiranecarbonylamino)-butyric acid (2e). This compound was prepared using general procedure E to give the product as a light orange oil (100% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.35–7.32 (m, 5H), 5.10 (s, 2H), 4.20 (t, 1H, J = 7.0 Hz), 3.34 (t, 1H, J = 4.6 Hz), 3.28 (t, 2H, J = 7.2 Hz), 2.85 (d, 2H, J = 4.6 Hz), 2.09 (td, 2H, J = 7.0 Hz, J = 7.1 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.52, 171.66, 171.58, 158.61, 138.08, 129.63, 129.16, 128.93, 67.86, 53.13, 50.14, 47.74, 36.94, 32.16. IR ν_{max} (cm^{-1}) 3330 (OH); 1743, 1690, 1681 (C=O). MS (FAB+) 323.1 (MH+).

N_α-Carbobenzyloxy-L-2-amino-5-(oxiranecarbonylamino)-pentanoic acid (3e). This compound was prepared using general procedure E to give the product as a soft-white powder (100% yield, mp 102 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.37–7.29 (m, 5H), 5.09 (s, 2H), 4.21 (t, 1H, J = 7.1 Hz), 3.36 (t, 1H, J = 4.8 Hz), 3.26 (t, 2H, J = 7.1 Hz), 2.86 (d, 2H, J = 4.7 Hz), 1.85–1.42 (m, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.18, 171.62, 158.70, 137.98, 129.72, 129.28, 128.98, 67.90, 55.19, 50.17, 47.85, 39.70, 30.03, 26.79. IR ν_{max} (cm^{-1}) 3332 (OH); 1741, 1698, 1683 (C=O). MS (FAB+) 337.1 (MH+).

N_α-Carbobenzyloxy-L-2-amino-6-(oxiranecarbonylamino)-hexanoic acid (4e). This compound was prepared using general procedure E to give the product as a white powder (100% yield, mp 95 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.37–7.27 (m, 5H), 5.10 (s, 2H), 4.22 (t, 1H, J = 7.0 Hz), 3.33 (t, 1H, J = 4.9 Hz), 3.25 (t, 2H, J = 6.9 Hz), 2.82 (d, 2H, J = 4.9 Hz), 1.85–1.38 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.36, 171.70, 158.34, 138.13, 129.58, 129.13, 128.94, 67.78, 56.32, 50.03, 47.51, 39.82, 32.96, 29.91, 23.97. IR ν_{max} (cm^{-1}) 3312 (OH); 1744, 1701, 1689 (C=O). MS (FAB+) 351.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-3-(oxiranecarbonylamino))propionylglycine *tert*-butyl ester (1f). This compound was prepared using general procedure E to give the product as a white powder (100% yield, mp 80 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.30 (m, 5H), 6.80 (s, 1H), 6.74 (s, 1H), 5.91 (s, 1H), 5.10 (s, 2H), 4.10 (t, 1H, J = 7.0 Hz), 3.84 (s, 2H), 3.46 (t, 1H, J = 4.5 Hz), 3.02 (d, 2H, J = 7.1 Hz), 2.79 (d, 2H, J = 4.6 Hz), 1.48 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.96, 168.96, 168.80, 156.46, 136.19, 128.41, 128.02, 127.86, 82.03, 66.88, 49.22, 46.96, 41.98, 41.87, 27.91, 27.68. IR ν_{max} (cm^{-1}) 1761, 1748, 1680, 1663 (C=O). MS (FAB+) 422.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-4-(oxiranecarbonylamino))butyrylglycine *tert*-butyl ester (2f). This compound was prepared using general procedure E to give the product as a white powder (100% yield, mp 118 °C). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.34–7.29 (m, 5H), 6.79 (s, 1H), 6.72 (s, 1H), 5.90 (s, 1H), 5.09 (s, 2H), 4.10 (t, 1H, J = 7.0 Hz), 3.84 (s, 2H), 3.45 (t, 1H, J = 4.6 Hz), 3.08 (t, 2H, J = 7.1 Hz), 2.77 (d, 2H, J = 4.6 Hz), 1.90 (td, 2H, J = 7.0 Hz, J = 7.1 Hz), 1.46 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.96, 169.84, 168.71, 156.34, 136.21, 128.41, 128.02, 127.83, 81.98, 66.84, 52.21, 49.34, 47.15, 41.93, 35.45, 32.97, 27.93. IR ν_{max} (cm^{-1}) 1752, 1747, 1684, 1675 (C=O). MS (FAB+) 436.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-5-(oxiranecarbonylamino))pentanoylglycine *tert*-butyl ester (3f). This compound was prepared using general procedure E to give the product as a dark yellow oil (100% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35–7.30 (m, 5H), 6.79 (s, 1H), 6.73 (s, 1H), 5.98 (s, 1H), 5.07 (s, 2H), 4.11 (t, 1H, J = 7.1 Hz), 3.84 (s, 2H), 3.46 (t, 1H, J = 4.5 Hz), 3.10 (t, 2H, J = 7.0 Hz), 2.79 (d, 2H, J = 4.5 Hz), 1.90–1.41 (m, 13H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.06, 169.36, 170.30, 156.35, 136.10, 128.55, 128.10, 127.94, 82.86, 67.76, 55.95, 49.99, 47.50, 42.83, 39.49, 30.63, 28.41, 26.73. IR ν_{max} (cm^{-1}) 1755, 1745, 1680, 1672 (C=O). MS (FAB+) 450.3 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-6-(oxiranecarbonylamino))hexanoylglycine *tert*-butyl ester (4f). This compound was prepared using general procedure E to give the product as a light orange oil (100% yield). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33–7.29 (m, 5H), 6.81 (s, 1H), 6.75 (s, 1H), 5.86 (s, 1H), 5.09 (s, 2H), 4.15 (t, 1H, J = 7.0 Hz), 3.83 (s, 2H), 3.42 (t, 1H, J = 4.6 Hz), 3.09 (t, 2H, J = 7.0 Hz), 2.82 (d, 2H, J = 4.6 Hz), 1.87–1.35 (m, 15H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.38, 168.79, 168.64, 156.24, 136.09, 128.25, 127.87, 127.72, 81.84, 66.66, 54.43, 49.21, 47.05, 41.75, 38.21, 31.80, 28.50, 27.76, 22.26. IR ν_{max} (cm^{-1}) 1760, 1749, 1682, 1667 (C=O). MS (FAB+) 464.4 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-3-(oxiranecarbonylamino))propionylglycine (1g). This compound was prepared using general procedure E to give the product as a light-yellow oil (100% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.39–7.33 (m, 5H), 5.13 (s, 2H), 4.11 (t, 1H, J = 6.6 Hz), 3.81 (s, 2H), 3.35 (t, 1H, J = 4.6 Hz), 3.20 (d, 2H, J = 6.6 Hz), 2.79 (d, 2H, J = 4.6 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 172.86, 172.10, 171.79, 158.24, 137.99, 129.62, 129.20, 129.06, 67.97, 52.95, 50.11. 47.74, 42.07, 41.18. IR ν_{max} (cm^{-1}) 3320 (OH); 1751, 1718, 1682, 1660 (C=O). MS (FAB+) 366.1 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-4-(oxiranecarbonylamino))butyrylglycine (2g). This compound was prepared using general procedure E to give the product as a light-yellow powder (100% yield, mp 111 °C). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.38–7.32 (m, 5H), 5.12 (s, 2H), 4.13 (t, 1H, J = 6.7 Hz), 3.79 (s, 2H), 3.28 (t, 1H, J = 4.5 Hz), 3.23 (t, 2H, J = 6.6 Hz), 2.81 (d, 2H, J = 4.6 Hz), 2.12 (td, 2H, J = 6.5 Hz, J = 6.5 Hz); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.82, 171.76, 171.56, 158.34, 138.03, 129.57, 129.13, 128.97, 67.90, 54.12, 50.06, 47.59, 41.96, 36.80, 32.95. IR ν_{max} (cm^{-1}) 3333 (OH); 1750, 1717, 1686, 1674 (C=O). MS (FAB+) 380.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-5-(oxiranecarbonylamino))pentanoylglycine (3g). This compound was prepared using general procedure E to give the product as a dark-yellow oil (100% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.35–7.30 (m, 5H), 5.08 (s, 2H), 4.15 (t, 1H, J = 6.5 Hz), 3.82 (s, 2H), 3.39 (t, 1H, J = 4.2 Hz), 3.19 (t, 2H,

$J = 6.6$ Hz), 2.88 (d, 2H, $J = 4.3$ Hz), 1.89–1.35 (m, 4H); ^{13}C NMR(CD_3OD, 100 MHz) δ 175.26, 171.78, 171.44, 158.35, 138.07, 129.58, 129.14, 128.95, 67.81, 55.97, 50.05, 47.58, 42.04, 41.94, 30.63, 26.67. IR ν_{max} (cm^{-1}) 3335 (OH); 1745, 1725, 1677, 1669 (C=O). MS (FAB+) 394.2 (MH+).

(N_α-Carbobenzyloxy-L-2-amino-6-(oxiranecarbonylamino))hexanoylglycine (4g). This compound was prepared using general procedure E to give the product as a light-yellow oil (100% yield). ^{1}H NMR (CD_3OD, 400 MHz) δ 7.36–7.33 (m, 5H), 5.09 (s, 2H), 4.15 (t, 1H, $J = 6.8$ Hz), 3.80 (s, 2H), 3.32 (t, 1H, $J = 4.3$ Hz), 3.20 (t, 2H, $J = 6.5$ Hz), 2.78 (d, 2H, $J = 4.5$ Hz), 1.92–1.37 (m, 6H); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.36, 171.70, 171.18, 158.34, 138.13, 129.58, 129.13, 128.94, 67.78, 56.32, 50.03, 47.51, 41.93, 39.82, 32.96, 29.91, 23.97. IR ν_{max} (cm^{-1}) 3340 (OH); 1750, 1720, 1680, 1670 (C=O). MS (FAB+) 410.2 (MH+).

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