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Design, Synthesis and Biological Evaluation of Succinimide Derivatives as Potential Mechanism-Based Inhibitors of Human Leukocyte Elastase, Cathepsin G and Proteinase 3

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Abstract—A structure–activity relationship study and *in vitro* biochemical studies with human leukocyte elastase, cathepsin G and proteinase 3 were conducted using a series of succinimide derivatives.

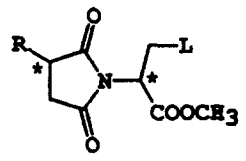
Introduction

The neutrophil-derived serine proteinase elastase, cathepsin G and proteinase 3 have been implicated in the pathogenesis of an array of chronic and acute inflammatory diseases.[1,2] The development of potent and specific low molecular weight inhibitors of these enzymes might lead to a better understanding of the pathophysiology of these diseases and the emergence of effective therapeutic modalities.[3,4]

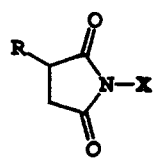
We have recently reported the results of synthetic and *in vitro* biochemical studies related to the application of the Gabriel–Colman rearrangement in the design of mechanism-based inhibitors of human leukocyte elastase (HLE) and cathepsin G (Cath G).[5] We present herein the results of our investigations related to the inhibition of elastase, cathepsin G and proteinase 3 by amino acid-derived succinimide derivatives (**I, II**).

Chemistry

Succinimide derivatives **1–3** and **5** were synthesized by reacting racemic 3-benzyl or racemic 3-isobutyl succinic acid, the appropriate amino acid, carbonyl diimidazole and triethylamine in tetrahydrofuran. Compounds **6**, **8** and **9** were prepared from the corresponding 3-alkyl substituted anhydrides and the appropriate amino acid esters. Refluxing racemic 3-benzylsuccinic anhydride with *S*-benzyl-L-cysteine methyl ester hydrochloride in the presence of triethylamine, followed by the addition of carbonyl diimidazole yielded compound **10**. The diastereomers **18–21** of compound **10** were prepared according to Scheme 1. Peracid oxidation of the precursor sulfides yielded compounds **7**, **11** and **12**. Compound **14** was obtained during the synthesis of **6**, and compound **13** was formed when **1** was treated with DAST in an attempted synthesis of the fluoro derivative (**I**, L = F, R = benzyl). Compounds **16** and **17** were obtained by alkylating 3-benzyl (or isobutyl) succinimide with sodium hydride/ethyl (α-bromomethyl)acrylate. All synthesized compounds, and their physical and spectral data are listed in Tables 1–3.



(I)



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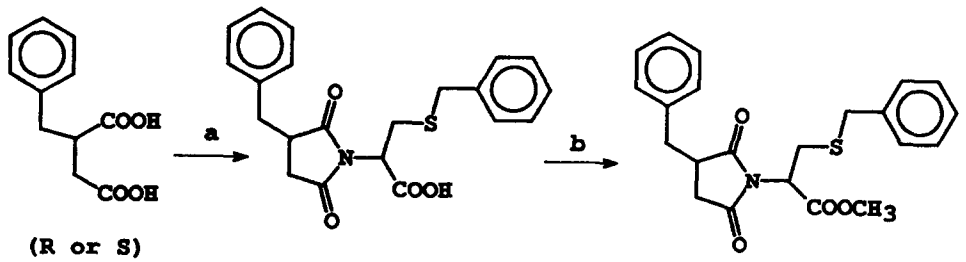


[a]S-benzyl-S or R-cysteine/TEA
carbonyl diimidazole/THF/Heat
[b]trimethylsilyldiazomethane/CH_3OH/benzene

Scheme 1.

Table 1. Derivatives of compound I

Compound	R	L
1	(*RS)-benzyl	(*S)-OH
2	(*RS)-benzyl	(*S)-OH[a]
3	(*RS)-benzyl	(*RS)-SH[b]
4	(*RS)-benzyl	(*RS)-$OPO(OEt)_2$
5	(*RS)-isobutyl	(*RS)-OH
6	(*RS)-isopropylthio	(*RS)-OH
7	(*RS)-isopropylsulfonyl	(*RS)-OH
8	(*RS)-isopropylthio	(*RS)-imidazole
9	(*RS)-isopropylthio	(*S)-SBzl
10	(*RS)-benzyl	(*S)-SBzl
11	(*RS)-benzyl	(*S)-SOBzl
12	(*RS)-benzyl	(*S)-SO_2Bzl

[a]benzyl ester; [b]acid

Table 2. Derivatives of compound II

Compound	R	X
13	(RS)-benzyl	$C(=CH_2)COOCH_3$
14	(RS)-isopropylthio	"
15	(RS)-isopropylsulfonyl	"
16	(RS)-benzyl	$CH_2C(=CH_2)COOCH_3$
17	(RS)-isobutyl	"

Biochemical Studies

The enzyme assays and inhibition studies were carried out as described in detail elsewhere.[6-8]

Results and Discussion

Based on some recent findings related to the inhibition of HLE, Cath G and PR 3 by a range of heterocyclic mechanism-based inhibitors,[9-13] we speculated that compounds represented by structure I might function as inhibitors of these enzymes. It was anticipated that catalytic processing of I by a target proteinase would result in unmasking the inherent latent reactive moiety in I and, ultimately, lead to irreversible inactivation of the enzyme as illustrated in Figure 1.

Surprisingly, compounds 1–12 were not found to inhibit HLE in a time-dependent manner. However, compounds 3 and 5 were found to be weak competitive inhibitors of HLE. The K_I values for compounds 3 and 5 were determined to be 0.5 and 0.18 mM, respectively. An assessment of the interaction of the rest of the compounds could not be made because of their low aqueous solubility. Varying the nature of the leaving group (L = OH, SH, $S(O)_nBzl$, $OPO(OEt)_2$,imidazole) or the nature of the R group (the primary specificity group believed to be accommodated at the S_1 subsite of the enzyme) also yielded inactive compounds. The introduction of the isopropylsulfonyl group, previously shown to optimize inhibitory activity in a related series of succinimide derivative,[14] did not produce the expected result. Interestingly, derivatives incorporating in their structure an unmasked conjugated system 13–17 were also found to be inactive.

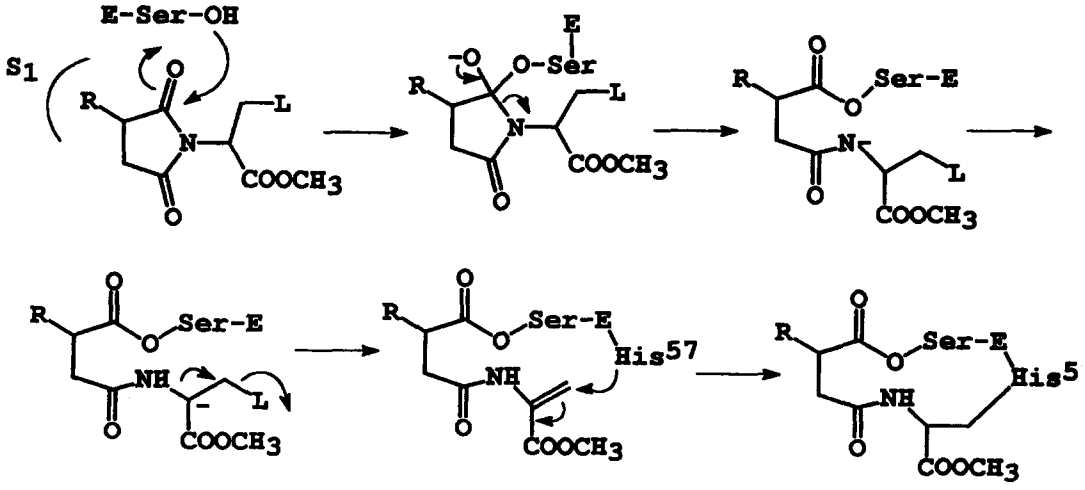


Figure 1. Postulated mechanism action of compound I.

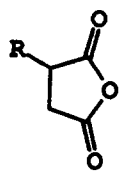
Table 3. Physical and spectral properties of compounds 1–17

Compound	MP °C	NMR ppm	MF (anal)
1	oil	2.5(dd,1H),2.78(dd,1H),2.95(m,1H), 3.22(m,2H),3.5(br,1H),3.73-3.77 (s,3H),4.04(m,2H),4.82(m,1H), 7.25(m,5H)	$C_{15}H_{17}NO_5$ C,H,N
2	oil	2.45(dd,1H),2.75(m,2H),3.2(m,2H),3.5 (br,1H),4.07(m,2H),4.87(m,1H),5.15 (s,2H),7.2(m,10H)	$C_{21}H_{21}NO_5$ C,H,N
3	70-5	2.5(m,1H),2.85(m,2H),3.22(m,2H), 3.75(m,2H),4.8(m,1H),7.25 (m,5H)	$C_{14}H_{15}NO_4S$ C,H,N
4	oil	1.3(m,6H),2.5(m,1H),2.73(dd,1H), 2.9(dd,1H),3.25(m,2H),3.7-3.75 (s,3H),4.08(m,4H),4.6(m,2H),5.05 (dd,1H),7.25(m,5H),	$C_{19}H_{26}NO_8P$ C,H,N
5	oil	0.98(m,6H),1.45(m,1H),1.8(m,2H), 2.48(m,1H),2.95(m,2H),3.52(br,1H), 3.78(s,3H),4.1(m,2H),4.85(m,1H)	$C_{12}H_{19}NO_5$ C,H,N
6	oil	1.25-1.40(dd,6H),2.57-2.65(dd,1H), 3.2-3.31(dd,1H),3.35-3.52(m,2H),3.75 (s,3H),3.9(dd,1H),4.10(d,2H),4.85(t,1H)	$C_{11}H_{17}NO_5S$ C,H,N
7	oil	1.41-1.58 (dd, 6H), 3.1-3.2 (m, 1H), 3.3-3.45 (m,1H),3.78(s,3H),3.80 (m,1H), 4.12 (dd, 2H), 4.50 (m, 1H), 4.90(m,1H)	$C_{11}H_{17}NO_7S$ C,H,N
8	oil	1.2-1.4(dd,6H),2.41-2.5(dd,1H), 3.02-3.2(dd,1H),3.32-3.40(m,1H), 3.7-3.8(m,1H),3.82(s,3H),4.68(d,2H), 5.05(dd,1H),6.9(d,1H),7.02(d,1H), 7.40(d,1H)	$C_{14}H_{19}N_3O_4S$ C,H,N
9	oil	1.30-1.42(dd,6H),2.58-2.67(m,1H), 3.12-3.29(m,3H),3.45(m,1H),3.7(d,2H), 3.75(s,3H),3.85(m,1H),4.85(m,1H), 7.35(m,5H)	$C_{18}H_{23}NO_2S_2$ C,H,N
10	oil	2.4-2.6(dd,1H),2.8-3.2(m,5H),3.3-3.4 (m,1H),3.6-3.7(d,3H),3.8(d,2H),4.9-5.0 (m,1H),7.2-7.4(m,10H)	$C_{22}H_{23}NO_4S$ C,H,N
11	oil	2.4-2.5(dt,1H),2.7-2.8(m,1H),3.2-3.4 (m,4H),3.7(d,3H),4.0-4.1(m,2H),5.2 (dd,1H),7.2-7.4(m,10H)	$C_{22}H_{23}NO_5S$ C,H,N
12	61-2°	2.5(dt,1H),2.7-2.9(m,2H),3.2-3.3(m,2H), 3.65(m,2H),3.7(d,3H),4.3(m,2H),5.3(dd, 1H), 7.2-7.3(m,5H),7.4(brs,5H)	$C_{22}H_{23}NO_6S$ C,H,N
13	oil	2.55(dd,1H),2.8(dd,1H),2.95(dd,1H), 3.25 (m,2H),3.75(s,3H),5.78(s,1H),6.58 (s,1H), 7.25(m,5H)	$C_{15}H_{15}NO_4$ C,H,N
14	oil	1.3(d,3H),1.42(d,3H),2.65(dd,1H),3.3 (dd,1H),3.45(m,1H),3.80(s,3H),3.92 (dd,1H),5.91(s,1H),6.62(s,1H)	$C_{11}H_{15}NO_4S$ C,H,N
15	146-8°	1.5-1.6(dd,6H),3.2-3.3(dd,1H),3.45-3.55 (dd,1H),3.89(s,3H),3.92(m,1H),4.60 (dd,1H), 6.05(s,1H),6.78(s,1H)	$C_{11}H_{15}NO_6S$ C,H,N
16	oil	2.51-2.58(dd,1H),2.72-2.81(dd,1H), 2.94-3.03 (dd,1H),3.15-3.23(m,2H)	$C_{16}H_{17}NO_4$ C,H,N
17	oil	0.95-1.02(tt,6H),1.32-1.41(m,1H), 1.70-1.90 (m,2H),2.39-2.48(dd,1H), 2.86-2.95(m,2H), 3.78(s,3H),4.38(s,2H), 5.50(dd,1H),6.30 (dd,1H)	$C_{13}H_{19}NO_4$ C,H,N

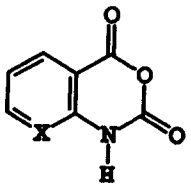
The lack of inhibitory activity of compounds **1–12** parallels that of the corresponding phthalimide and saccharin derivatives,[12] and may in part be related to the lower chemical reactivity (lower electrophilicity) of the carbonyl carbon.[15,16] For example, we have observed that the attachment of -CH_2COOR to a heterocyclic nucleus (phthalimide, saccharin, succinimide, isothiazolidin-3-one) yields inactive compounds. In contrast, the attachment of -CHFCOOEt gives rise to time-dependent inhibitors of the enzyme.[12]

During the course of these studies, it was observed that

the *crude* diastereomeric mixture formed during the synthesis of **10** showed time-dependent and transient inhibitory activity toward HLE. We surmised that the observed activity was due to one of the diastereomers and proceeded to synthesize pure diastereomers **18–21**. Subsequent screening of **18–21** revealed that all four diastereomers lacked inhibitory activity toward HLE. Indeed, further investigations showed that the active component in crude **10** was 3-benzyl-succinic anhydride and that, in general, 3-alkyl substituted anhydrides **22–24** function as substrates of HLE (Fig. 2).



R = isopropylthio 22
benzyl 23
n-butyl 24



X = CH 25
N 26

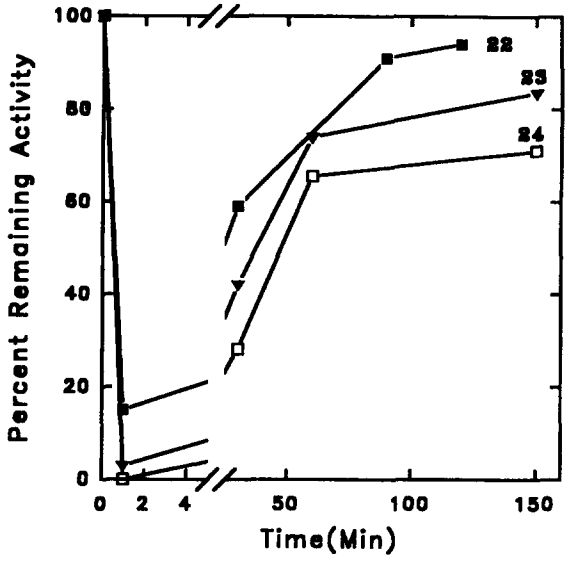


Figure 2. Time dependence of enzymatic activity. Human leukocyte elastase (395 nM) was incubated with anhydrides **22–24** (79 μM) in 0.1 M HEPES buffer, pH 7.25, 0.5 M NaCl and 1% dimethyl sulfoxide.

Anhydrides with built in features aimed at slowing the deacylation pathway such as, for example, isatoic anhydride (**25**) and 5-butyl-3*H*-1,3-oxazine-2,6-diones have been known to function as effective inhibitors of chymotrypsin and porcine pancreatic elastase.[17,18] We have found that the behavior of HLE toward isatoic anhydride (**25**) is similar to that of chymotrypsin, forming a relatively stable acyl enzyme. In contrast, the interaction of HLE with 7-azaisatoic anhydride (**26**) leads to the formation of an acyl enzyme that deacylates rapidly, effectively functioning as an alternate substrate of the enzyme (Fig. 3).

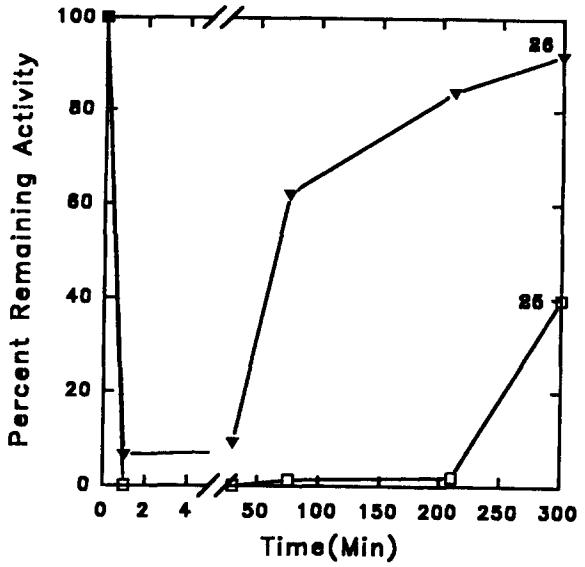


Figure 3. Time dependence of enzymatic activity. Human leukocyte elastase (348 nM) was incubated with anhydrides **25–26** (69.6 μM) in 0.1 M HEPES buffer, pH 7.25, 0.5 M NaCl and 1% dimethyl sulfoxide.

In conclusion, the interaction of succinimide derivatives and anhydrides toward HLE, Cath G and PR 3 has been investigated. Further studies aimed at gaining insight and understanding about the factors that determine the mode of interaction of these compounds with serine proteinases are currently in progress.

Experimental

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The IR and NMR spectra of the synthesized compounds were recorded on a Perkin-Elmer 1330 infrared spectrophotometer and a Varian XL-300 NMR spectrometer, respectively. A Gilford or HP UV/Vis spectrophotometer was used in the enzyme assays and inhibition studies. Human leukocyte elastase was purchased from Elastin Products Co. (Owensville, MO). Human leukocyte cathepsin G and proteinase 3 were obtained from Athens Research and Technology, Co. (Athens, GA). Methoxysuccinyl Ala-Ala-Pro-Val *p*-nitroanilide, methoxysuccinyl Ala-Ala-Pro-Phe *p*-nitroanilide and Boc-L-Ala *p*-nitrophenol were purchased from Sigma Chemical Co. (St. Louis, MO). The IUPAC names of the synthesized compounds were obtained using AUTONOM 1.1 (Beilstein Informationssysteme GmbH, Frankfurt, Germany).

Methyl 1-(3-hydroxy-2-propanoate)-3-benzyl-2,5-pyrrolidine dione (1). Carbonyl diimidazole (3.58 g; 22 mmol) and racemic 2-benzyl succinic acid (2.08 g, 10 mmol) were dissolved in 30 mL dry THF under an argon atmosphere and refluxed for 20 min. The solution was cooled to room temperature and triethylamine (1.01 g; 10 mmol) and L-serine methyl ester (1.56 g, 10 mmol) were added sequentially. The reaction mixture was stirred at room temperature for 14 h. The solvent was evaporated and the residue was taken up in EtOAc and

washed with ice-cold 3% HCl, 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. Evaporation of the solvent left 2.16 g of a crude oil which was purified by flash chromatography, yielding 360 mg (12% yield) of pure **1** as a colorless oil.

Benzyl 1-(3-hydroxy-2-propanoate)-3-benzyl-2,5-pyrrolidine dione (2). This was prepared using a similar procedure to that used in the preparation of **1**, using (L)-serine benzyl ester. The crude product (4.13 g, 94% yield) was purified by flash chromatography, yielding a pure, colorless oil (620 mg; 14% yield).

1-(3-Mercapto-2-propanoic acid)-3-benzyl-2,5-pyrrolidine dione (3). Carbonyl diimidazole (3.41 g; 21 mmol) and racemic 2-benzyl succinic acid (2.0 g; 9.6 mmol) were dissolved in 30 mL dry THF under an Ar atmosphere and refluxed for 20 min. The solution was cooled to room temperature and triethylamine (0.97 g, 9.6 mmol), DMF (10 mL) and DL-cysteine (1.16 g, 9.6 mmol) were added sequentially. The heterogenous reaction mixture was stirred at 40 °C for 1.5 h then cooled to room temperature and stirred overnight. The solvent was evaporated and the residue was taken up in EtOAc and acidified with 5% HCl. The organic layer was washed with 5% HCl, H_2O and dried. Evaporation of the solvent left a crude product (2.42 g, 86% yield) which was purified by flash chromatography (350 mg, 13% yield).

Methyl 1-(3-diethylphosphono-2-propanoate)-3-benzyl-2,5-pyrrolidine dione (4). Dry pyridine (1.07 g, 13.5 mmol) and compound **1** (1.0 g, 3.4 mmol) were dissolved in dry THF (10 mL) and DMF (6 mL) and cooled to -65 °C under an argon atmosphere. Diethyl chlorophosphite (0.7 g, 4.5 mmol) was introduced into the cooled solution via syringe and the solution was stirred for 25 min at -55 °C. A solution of iodine (1.39 g, 5.44 mmol) in 1 mL water and 7 mL THF was then added dropwise to the solution at such a rate as to maintain the temperature at -50 °C. The solution was allowed to warm up to 0 °C, while being stirred over a period of 20 min. A 20% $NaHSO_3$ solution (10 mL) was added, followed by the addition of EtOAc (50 mL) and H_2O (10 mL). The layers were separated and the aqueous layer was washed once with EtOAc and the combined organic extracts were washed with 5% HCl, H_2O and dried over anhydrous Na_2SO_4. Removal of the solvent yielded 1.34 g (92%) of a crude oil which was purified by flash chromatography (740 mg, 51% yield).

Methyl 1-(3-hydroxy-2-propanoate)-3-isobutyl-2,5-pyrrolidine dione (5). Carbonyl diimidazole (5.35 g, 33 mmol) and racemic 2-isobutyl succinic acid (2.5 g, 15 mmol) were dissolved in 50 mL dry THF under Ar atmosphere and stirred at room temperature for 1 h. Triethylamine (1.52 g, 15 mmol) and DL-serine methyl ester (2.33 g, 15 mmol) were added sequentially to the solution. The reaction mixture was stirred at room temperature for 18 h. The solvent was evaporated and the residue was taken up in EtOAc and washed with 5% HCl, 5% $NaHCO_3$, H_2O and dried over anhydrous Na_2SO_4. Removal of the solvent left a crude oil (1.55 g, 40% yield) which was purified by flash chromatography (290 mg, 8% yield).

Methyl 1-(3-hydroxy-2-propanoate)-3-isopropylthio-2,5-pyrrolidine dione 6 and 2-(3-isopropylsulfanyl-2,5-pyrrolidin-1-yl) acrylic acid methyl ester (14). A mixture of DL-serine methyl ester hydrochloride (2.33 g, 15 mmol) and triethylamine (1.52 g, 15 mmol) in 50 mL dry methylene chloride was stirred for 20 min at room temperature. The temperature was raised to 45 °C using a water bath and racemic 3-(isobutylthio) succinic anhydride (2.6 g, 15 mmol) in 75 mL dry THF was added. The reaction mixture was kept at 45 °C for 45 min. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (1.52 g, 8 mmol) and 30 mL of CH_2Cl_2 were added and the solution was refluxed overnight. The solvent was removed and the residue was taken up in EtOAc. The organic extract was washed with H_2O, dried and evaporated to yield a crude product which was purified by flash chromatography, yielding **6** (36% yield) and **14** (12% yield).

Methyl 1-(3-hydroxy-2-propanoate)-3-isopropylsulfonyl-2,5-pyrrolidine dione (7). Compound **6** (0.6 g, 2.7 mmol) in CH_2Cl_2 (30 mL) was treated with *m*-chloroperbenzoic acid (0.94 g). The reaction mixture was stirred overnight. The precipitate was filtered off and the solvent evaporated *in vacuo*, leaving a crude product which was purified by flash chromatography, yielding **7** (0.30 g, 45% yield).

Methyl 1-(3-imidazolyl-2-propanoate)-3-isopropylthio-2,5-pyrrolidine dione (8). A mixture of DL-serine methyl ester hydrochloride (2.33 g, 15 mmol) and triethylamine (1.52 g, 15 mmol) in 50 mL methylene chloride was stirred for 20 min. The solvent was removed on the rotovac and the residue was dissolved in THF (75 mL). 3-(Isopropylthio)succinic anhydride (2.6 g, 15 mmol) was added, the temperature was raised to 45°C and stirring was continued for 0.5 h. The solution was cooled to room temperature and carbonyl diimidazole (4.86 g, 30 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated off and the residue was taken up in EtOAc (100 mL). The organic layer was washed with water and 3% $NaHCO_3$. The solvent was dried and evaporated, yielding a crude product which was purified by flash chromatography (2.27 g, 47% yield).

Methyl 1-(3-benzylthio-2-propanoate)-3-isopropylthio-2,5-pyrrolidine dione (9). Triethylamine (1.52 g) was added to *S*-benzyl-L-cysteine methyl ester hydrochloride (3.93 g, 15 mmol) in dry CH_2Cl_2 (20 mL). The solution was stirred for 15 min at room temperature. Dry THF (50 mL) was then added, followed by 3-(isopropylthio)succinic anhydride (2.61 g, 15 mmol). The temperature was raised to 40 °C and the mixture stirred for 0.5 h. The solution was cooled to room temperature, carbonyl diimidazole (2.67 g) was added and stirring was continued overnight. The solvent was evaporated and the residue taken up in Et_2O (75 mL). The organic

layer was washed with water, dried and evaporated, leaving pure **9** (5.57 g, 94% yield) as a colorless oil.

Methyl 1-(3-benzylthio-2-propanoate)-3-benzyl-2,5-pyrrolidine dione (10). *S*-Benzyl-L-cysteine methyl ester hydrochloride (4.0 g; 15 mmol) was added to a solution of 3-benzylsuccinic anhydride (3.0 g, 15 mmol) in freshly distilled tetrahydrofuran (100 mL), followed by the addition of triethylamine (1.5 g, 15.2 mmol). The reaction mixture was refluxed for 20 min, and then cooled to room temperature Carbonyl diimidazole (3.0 g; 18 mmol) was added and the reaction mixture was stirred overnight at room temperature. Removal of the solvent *in vacuo* left a residue which was taken up in CH_2Cl_2 (100 mL) and washed with H_2O (25 mL), 5% aqueous HCl, and dried over anhydrous Na_2SO_4. Evaporation of the solvent left a crude oil which was purified by flash chromatography (5.0 g, 84% yield).

2-(3-Benzyl-2,5-dioxo-pyrrolidin-yl)-3-phenylsulfinyl-propionic acid methyl ester (11). *m*-Chloroperbenzoic acid (0.72 g; 4.2 mmol) was added to a solution of methyl 1-{3(*S*)-benzylthio-2-propanoate}-3(*RS*)-benzyl 2,5-pyrrolidine dione (1.5 g, 3.8 mmol) in 50 mL CH_2Cl_2 and the reaction mixture was allowed to stir overnight at room temperature. CH_2Cl_2 (50 mL) and H_2O (60 mL) were added and the layers were separated. The organic layer was washed with 5% $NaHCO_3$ and dried. Removal of the solvent gave a crude product which was purified by flash chromatography using CH_2Cl_2–EtOAc as eluents (1.0 g, 64% yield).

2-(3-Benzyl-2,5-dioxo-pyrrolidin-1-yl)-3-phenylsulfonyl-propionic acid methyl ester (12). *m*-Chloroperbenzoic acid (2.4 g, 13.8 mmol) was added to a solution of methyl 1-{3(S)-benzylthio-2-propanoate}-3(*RS*)-benzyl 2,5-pyrrolidine dione (2.2 g, 5.5 mmol) in CH_2Cl_2 (60 mL). Work-up as for **14** gave a crude product which was purified by flash chromatography using CH_2Cl_2 as the eluent (1.4 g, 60% yield).

Methyl 1-(2-propanoate)-3-benzyl-2,5-pyrrolidine dione (13). Compound **1** (1.0 g, 3.4 mmol) in 10 mL dry THF was added dropwise to a solution of diethylaminosulfur trifluoride (DAST) (0.68 g, 4.08 mmol) in 10 mL dry THF under an argon atmosphere. The reaction mixture was stirred for 3 h at room temperature. H_2O (30 mL) was added cautiously to the solution and extracted with EtOAc. The organic layer was washed with H_2O, dried and evaporated to yield a crude product which was purified by flash chromatography, yielding 335 mg (37% yield) of **13** as a colorless oil.

Methyl 1-(2-propanoate)-3-isopropylsulfonyl-2,5-pyrrolidine dione (15). Sulfide **14** (0.23 g, 0.9 mmol) in CH_2Cl_2 (30 mL) was treated with *m*-chloroperbenzoic acid (0.17 g, 10 mmol). The reaction mixture was stirred overnight. CH_2Cl_2 (100 mL) was added and the solution was washed with 2% Na_2CO_3 and dried. Evaporation of the solvent left pure **15** (0.15 g, 60% yield).

2-(3-Benzyl-2,5-dioxo-pyrrolidin-1-ylmethyl)-acrylic acid methyl ester (16). A solution of 3-benzylsuccinimide (2.4 g, 12.7 mmol) in dry DMF (15 mL) was added to an ice-cold mixture of NaH (0.39 g, 16.5 mmol) in 20 mL of dry DMF with stirring. The reaction mixture was allowed to warm to room temperature and stirred for 20 min. Ethyl(α-bromomethyl)acrylate (2.88 g, 15 mmol) was added dropwise and stirring was continued for 3 h. Most of the DMF was removed *in vacuo* and the residue was treated with saturated NH_4Cl (30 mL). The solution was extracted with EtOAc. Evaporation of the solvent left a crude product which was purified by flash chromatography (1.02 g, 30% yield).

2-(3-Isobutyl-2,5-dioxo-pyrrodin-1-ylmethyl)acrylic acid methyl ester (17). A solution of 3-isobutylsuccinimide (2.17 g, 14 mmol) in 15 mL dry DMF was added to an ice-cold mixture of NaH (0.4 g, 17.3 mmol) in 20 mL dry DMF with stirring. Ethyl(α-bromomethyl)acrylate (2.51 g, 14 mmol) was added dropwise and stirring was continued for 3 h. Most of the DMF was removed *in vacuo* and the residue was treated with saturated Na_4Cl (30 mL). The solution was extracted with EtOAc, dried and evaporated. The crude residue was purified by flash chromatography, yielding 1.5 g (42%) of compound **17**.

Methyl 1-(3-benzylthio-2-propanoate)-3-benzyl-2,5-pyrrolidine dione (18–21). Carbonyl diimidazole (2.0 g, 12 mmol) was added portionwise to a solution of *R* (or *S*)-2-benzylsuccinic acid (1.0 g, 4.8 mmol) (**11**) in anhydrous THF (20 mL). The reaction mixture was then refluxed for 20 min and cooled to room temperature. Dry triethylamine (0.48 g, 4.8 mmol) was added, followed by the addition of *S*-benzyl-(*S*) or (*R*)-cysteine (1.0 g, 4.8 mmol). The reaction mixture was brought to reflux for 1 h and concentrated *in vacuo*. The residue was taken up in EtOAc (80 mL) and washed with 5% aqueous HCl. Removal of the solvent left a colored oil which was purified on a chromatotron plate using EtOAc:MeOH (5:1) as eluent. Each diastereomeric acid was esterified according to the following procedure and without further purification. To a stirred solution of each diastereomeric carboxylic acid (0.9 g, 2.4 mmol) in 5 mL dry MeOH and 16 mL benzene was added trimethylsilyldiazomethane (1.5 mL in 2 M hexane, 3.05 mmol) in 2 mL benzene at room temperature. The reaction mixture was stirred at room temperature for another 30 min and concentrated to give a crude oil. This was purified on a chromatotron plate using CH_2Cl_2 as eluent, yielding diastereomeric esters **18–21**. (*S,R*)-isomer **18**: $[\alpha]^{25}_D$ +55.5 (*c* 1.7; MeOH); (*R,S*)-isomer **19**: $[\alpha]^{25}_D$ –56.5 (*c* 2.3, MeOH); (*S,S*)-isomer **20**: $[\alpha]^{25}_D$ 15.6 (*c* 1.2; MeOH); (*R,R*)-isomer **21**: $[\alpha]^{25}_D$ +19.5 (*c* 1.0; MeOH).

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