Preparation of Dicarboxylate Analogues of Cerulenin

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We have proposed and synthesized several new structural classes of Cerulenin analogues, which have potential as inhibitors of both fatty acid and polyketide synthase multi-enzyme complexes. These analogues contain *cis* epoxides bearing flanking carboxylate groups. Our syntheses have been designed to allow access to a wide range of fatty acid and polyketide-like side chains from readily available starting materials in convergent fashion in just four to five steps. In total, ~40 potential analogues have been prepared and characterized, covering all the structural sub-classes proposed, the majority of which constitute novel functional groupings.

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Introduction.

Cerulenin (**1**) is a broad-spectrum antibiotic that was first detected in the fermentation broth of *Cephalosporium caerulens* due to its potent antifungal properties [1]. It was quickly shown to be active against the fatty acid synthases (FAS) and polyketide synthases (PKS) of many fungi, yeast and bacteria [2]. Consequently, it has been greatly used as a biochemical tool in a wide range of biochemical studies [3], and not only limited to the biochemical elucidation of fatty acid and polyketide metabolic pathways [4].

Cerulenin's structure was finally confirmed in 1974 by Arison [5]. A number of total and formal syntheses, in both racemic and homochiral form, have been reported and these were comprehensively reviewed by Yoda in 1993 [6]. Since then three further homochiral syntheses have been reported by Hegedus [7], Townsend [8] and Ghosez [9]. An added complication for the synthesis of Cerulenin is that in protic solvents the open chain form **1** exists in equilibrium with its hydroxylactam form **2**, which is expected to predominate in biological systems [10] (Figure 1).

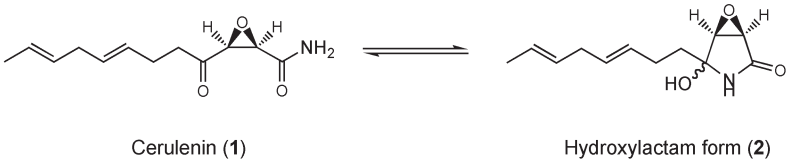


Figure 1. Interconversion of Cerulenin tautomers.

We were interested in Cerulenin as a potential tool for elucidating the mechanism of polyketide biosynthesis. Our ultimate aim was to substitute the unsaturated fatty acid-like side chain of Cerulenin with a tailor-made polyketide-like side chain designed for a specific biosynthesis, whilst retaining the inhibitory properties of Cerulenin. A number of studies of Cerulenin analogues have been reported, but the focus of the majority of these is on the role of the unusual 1,3-unsaturated side chain, and particularly its role in inhibiting FAS and other systems [10-12]. Biochemical studies have shown that the *cis*-epoxide flanked by two carbonyl groups is critical to Cerulenin's inhibitory activity [2f], in which a cysteine residue opens the epoxide at C-2 (**3**) [13], thus irreversibly deactivating the β-ketoacyl synthase (Figure 2).

In spite of the successful syntheses surveyed by Yoda [6], the all-carbon backbone of Cerulenin containing the dicarbonyl flanked *cis* epoxide functionality remains synthetically challenging. Syntheses are often lengthy, especially where the side chain is carried through the synthetic route. However, the more convergent syntheses [8-9, 14] could be amenable to the introduction of all-carbon analogues in a more efficient fashion as the authors propose, by addition of the C_8/C_9 side chains to homochiral C_4/C_3 "head unit" synthons (Scheme 1).

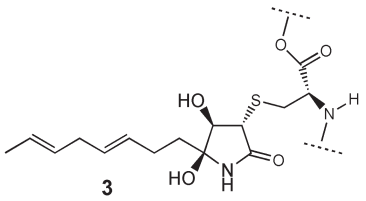
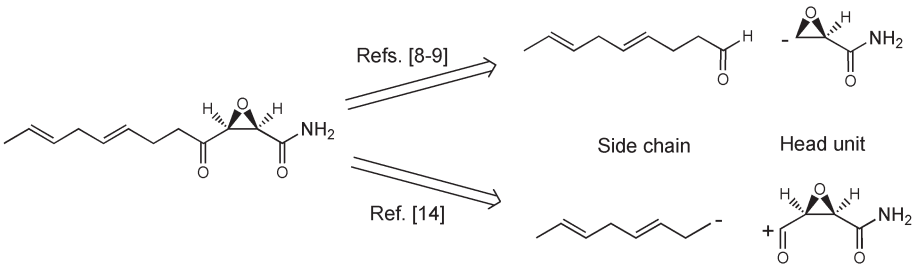


Figure 2. Cysteine opening of Cerulenin's epoxide functionality.

Scheme 1

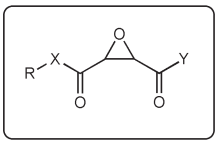


Retrosynthesis of Cerulenin.

We speculated that the C_4 head unit might be replaced by a series of similar functional groups based on dicarboxylates, which would be easier to synthesize. This was important as we expected that the potential polyketide-like side chains we required would be synthetically challenging in themselves. Addition of either simple or complex tailor-made side chains to these analogous head units would allow us quickly to explore the structure-activity relationships of Cerulenin analogues in a wide range of biologically interesting systems.

Therefore, we proposed the structures **4-12**, which retained the *cis* epoxide functionality and the flanking carbonyls groups, but with both carbonyls at the carboxylate oxidation level *i.e.* replacing the ketone with an ester (**4-6**), amide (**7-9**) or thioester (**10-12**) function (Figure 3). The syntheses of these compounds would be greatly simplified compared to their all-carbon analogues (**13**). To further increase the scope of analogue synthesis, the other carboxylate function was to be from a common intermediate. Thus we decided to prepare the carboxylic acids (**4**, **7**, **10**), [15] the methyl esters (**5**, **8**, **11**), and the carboxamides (**6**, **9**, **12**) in each series.

There is some debate as to which is the active form of Cerulenin, **1** or **2**, although this may be dependent on the individual biological system. Tishler's results on the 4-hydroxy analogues, including **14**, showed that the lack of a carbonyl group at C-4 destroyed the inhibitory activity [10]. This could be attributed to their inability to form the hydroxylactam tautomers. In contrast, the 4-keto analogues with a dimethyl amide, which also cannot form hydroxylactams, were active in Tishler's hands in both the cerulenin-like (**15**) and tetrahydrocerulenin (**16**) cases (but the cyclic amides pyrrolidine and piperidine were inactive) (Figure 4). However, a more comprehensive study by Lichtenhaler [12] of several 4-hydroxy and dimethylamide analogues with variable chain lengths including **16**, showed no activity against any plant FAS [16]. The only other analogue that has featured variation in the head unit is the carbocyclic analogue prepared by Iwasaki [17] (**17**), a deliberate attempt to mimic the hydroxylactam form **2**. This molecule, although a direct analogue of racemic Cerulenin with the 1,3-unsaturated side chain, was effectively inactive against yeast FAS, which tends to discount **2** as the active form. However, the authors also noted that the nitrogen atom may be important in this case.



Esters	Amides	Thioesters
4 (X = O, Y = OH)	**7** (X = NH, Y = OH)	**10** (X = S, Y = OH)
5 (X = O, Y = OMe)	**8** (X = NH, Y = OMe)	**11** (X = S, Y = OMe)
6 (X = O, Y = NH_2)	**9** (X = NH, Y = NH_2)	**12** (X = S, Y = NH_2)

13 (X = CH_2, Y = any)

Figure 3. Proposed dicarboxylate analogues of Cerulenin (target structures boxed in following schemes).

It was hoped that our analogues would help to resolve this question since they retained the *cis* epoxide with the flanking dicarbonyl motif, but would be unable to form the hydroxylactams [18]. Interestingly, whilst many analogues of **1** have been prepared and investigated [10-12,17], they have all been carbon analogues (**13**). Studies in these cases have usually focused on relatively simple analogues in the side chain [11,12], and variation in the head unit has been restricted to the relatively minor modifications of substituted amides discussed above [10,14]. With the exception of **17**, the dicarboxylate analogues **4-12** are the first significant examples of analogues of the head unit.

Results and Discussion.

The ester-acids (**4**) were readily prepared starting from the known epoxymaleic anhydride **19** (Scheme 2). Sodium tungstate catalysed epoxidation of maleic acid or its anhydride gave the disodium salt in 99% yield on a molar scale, which after conversion through its barium salt gave the

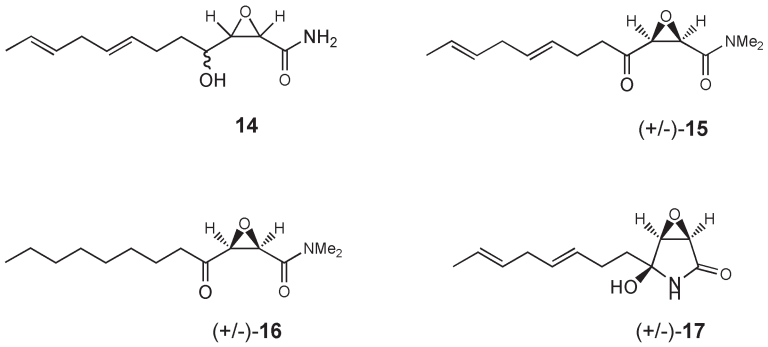
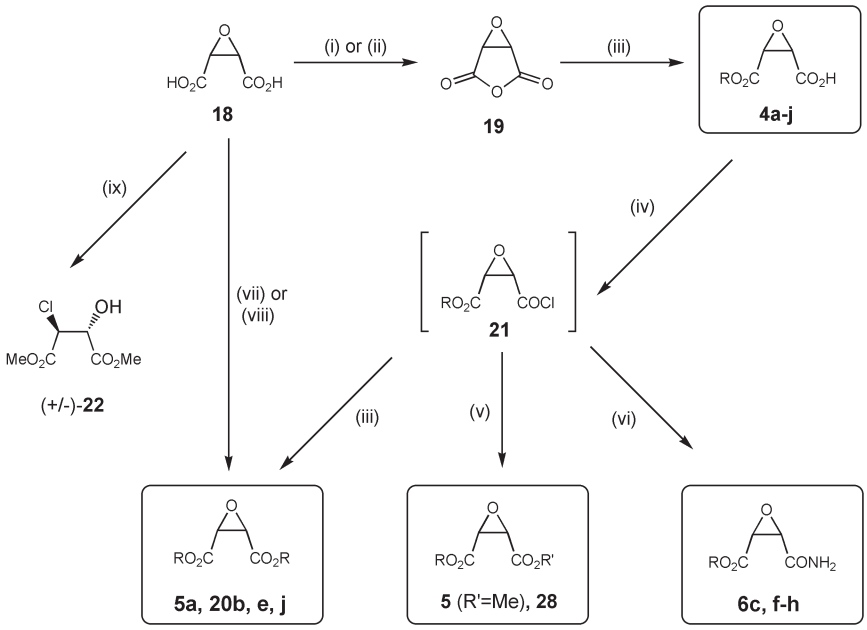


Figure 4. All-carbon analogues of Cerulenin.

free acid (**18**) in 75-80% yield overall [19]. The key anhydride **19** [20] was formed by stirring in neat trifluoroacetic anhydride [21] or, more economically, by refluxing **18** with 2 equivalents of TFAA in dichloromethane. Ring opening of **19** was achieved by dissolution in the appropriate neat alcohol to give the half ester-acids **4a-j** in near quantitative yields (Scheme 2 and Table 1). These were generally viscous oils or gums that were difficult to purify, as typified by the known **4a** [21,22]. However, the octyl (**4h**) and benzyl (**4j**) analogues did give crystalline products. Others were successfully used crude to give later intermediates. For example, both symmetrical (**20**) and unsymmetrical (**5**) diesters [23] could be prepared from these half ester-acids by activation with PCl_5 *via* **21**, and reaction with the appropriate alcohol.

The shorter chain symmetrical esters (**5a, 20b**) could also be prepared directly from **18** by acid-catalysed esterification [24]. Attempts to make the longer chain esters (*i.e.* >*i*Pr) led to ether formation instead, so PCl_5 activation of **18** *via* the diacid chloride (not shown) had to be used to prepare, for example, **20e** and **20j**. An alternative attempt to directly activate diacid **18** with $SOCl_2$ and quench with methanol led to formation of the chlorohydrin **22** instead. The carboxamide series (**6**) was prepared in a similar fashion, activating the ester-acids with PCl_5 and then quenching the intermediate acid chlorides (**21**) with NH_3 to give

Scheme 2



Reagents and conditions: (i) TFAA (neat), TFA (cat.); (ii) TFAA (2.0 eq.), CH_2Cl_2; (iii) ROH; (iv) PCl_5, CH_2Cl_2; (v) MeOH; (vi) NH_3 (g); (vii) ROH, H^+; (viii) PCl_5, py., ROH; (ix) $SOCl_2$, MeOH.

the ester-amides **6c** and **6f-h**. Yields were lower for the amide series, but adequate for our purposes. All three routes were short and ideal for analogue synthesis with the potential to add more complex side chains as a last step.

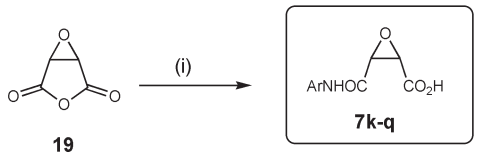
Table 1

Key for Generic R Group Substituent on all Schemes

a = Me	**f** = nHex	**k** = Ph	**p** = 2 x $PhCH_2$
b = Et	**g** = nHep	**l** = 4-$MeOC_6H_4$	**q** = 4-$MeOC_6H_4CH_2$
c = iPr	**h** = nOct	**m** = 4-$NO_2C_6H_4$	**r** = nPen
d = nBu	**i** = $PhCH_2CH_2$	**n** = 2,4-di$NO_2C_6H_3NH$	
e = tBu	**j** = $PhCH_2$	**o** = Ph_3C	**s-w** = as noted on schemes

Our second series of targets were the amide analogues (**7-9**). Ring opening of the anhydride **19** with aromatic amines was a known strategy [20]. Several anilines, a hydrazine, tritylamine and dibenzylamine all reacted smoothly and in good yields to give the highly crystalline aromatic amide-acids (**7k-p**) (Scheme 3). However, 4-methoxybenzylamine failed to react cleanly and its analogue **7q** could not be prepared. Simple aliphatic amines required more forcing conditions (overnight reflux in THF), and gave the desired amide-acids in fair yields but only moderate purity.

Scheme 3



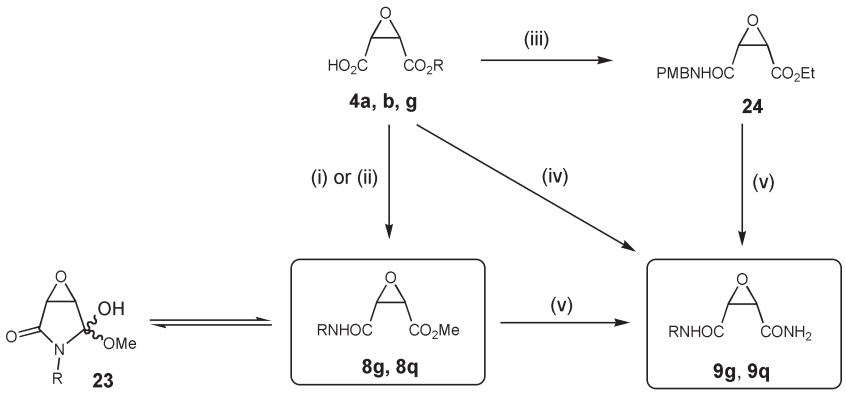
Reagents and conditions: (i) $ArNH_2$, Et_2O.

Activation of the amide-acids (**7**) with PCl_5, oxalyl chloride or DCC and then quenching of the intermediates with methanol gave moderate yields of the aliphatic amide-esters, which were difficult to isolate and purify (only **8g** characterized). The NMR data suggested they might co-exist in their methoxy-lactam forms (**23**) (Scheme 4). Furthermore, these methods failed altogether for the aromatic acids (**7k-p**), giving complex mixtures of brown gum; hygroscopicity was thought to be an issue. A better procedure for the aliphatic amide-esters **8**, and for the PMB example **8q** was to start from the ester-acids **4a** or crude **4b**, activate with DCC or PCl_5, and quench with the appropriate amine (Scheme 4; only **8g**, **8q** and **24** prepared). Simple treatment of **8q** with methanolic ammonia gave the PMB-diamide **9q**; in the alkyl series **9g** was prepared directly from **7g** instead. In view of the difficulties encountered with the synthesis of structures **8** and **9**, alternative routes to these classes of compounds were attempted which are discussed below. However, examples were prepared in each class of target molecule (**7-9**) as planned.

The third major group of target molecules was that of the thioesters **10-12**. Curiously, dissolution of anhydride **19** in neat thiol to give the thioester-acids **10** did not proceed as for the alcohols **4**, and none could usefully be prepared in pure form. Instead, ester-acids **4** were activated with DCC and quenched with stoichiometric thiol to give the thioester-esters (**11c, e, g, r, 25t, 25u**) in moderate to good yields (Scheme 5). Symmetrical dithioesters could be also prepared under these conditions directly from acid **18**, of which a single example **26** was prepared.

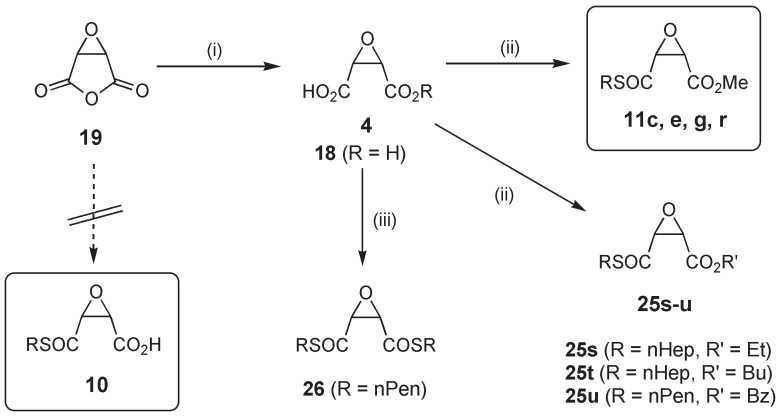
Since the thioester-acids **10** could not be prepared, the analogous strategy as that used for the amide-amides **9** (*cf.*

Scheme 4



Reagents and conditions: (i) **4a**, PCl_5, CH_2Cl_2, then heptylamine; (ii) **4a**, DCC, DMAP, CH_2Cl_2 then 4-PMB-NH_2; (iii) crude **4b**, PCl_5, CH_2Cl_2, then 4-PMB-NH_2; (iv) crude **4g**, PCl_5, CH_2Cl_2, then NH_3 (g); (v) NH_3 (aq), MeOH.

Scheme 5

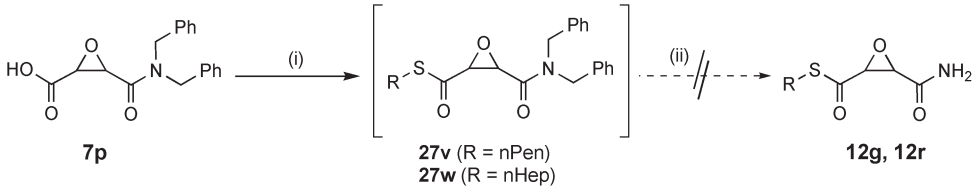


Reagents and conditions: (i) ROH; (ii) Ester-acid **4**, DCC, DMAP, THF, then RSH; (iii) **18**, DCC, DMAP, THF, then nPenSH.

Scheme 4) could not be used to make the thioester-amides **12** (attempts to use the crude thioester-acids did not succeed). Two nitrogen-protected thioester-amides (**27v**, **27w**) were prepared from dibenzyl amide-acid (**7p**) under standard conditions (DCC, RSH), but the benzyl groups could not be removed to give the respective primary carboxamides (**12g, 12r**) (Scheme 6) (presumably due to catalyst poisoning by thiol residues).

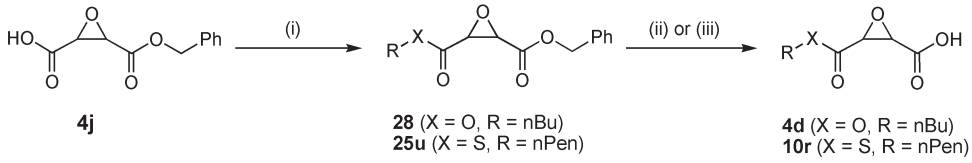
Alternatively, we tested the more facile removal of the O-benzyl ester from diester **28** which gave the desired crude half ester-acid **4d** in high yield without reduction of the epoxide (Scheme 7). Hydrogenolysis of the analogous thioester-ester **25u** would have given access to a thioester-acid **10r** from which a thioester-amide **12r** might have been prepared, but these reductions again failed, even with the use of stoichiometric "catalyst", and the more effective Pearlman's catalyst.

Scheme 6



Reagents and conditions: (i) DCC, DMAP, THF, then RSH; (ii) Pd/C, H_2.

Scheme 7



Reagents and conditions: (i) DCC, DMAP, THF, then RXH; (ii) **25u** or **28**, Pd/C, H_2, THF; (iii) **25u**, $Pd(OH)_2$, H_2, EtOH.

Finally, although we had shown that thioester tertiary-amides such as **27v** and **27w** could be prepared, we had failed to synthesize the primary amide versions (eg. **12g**, **12r**, Scheme 6), which were of special interest. Since removal of the dibenzyl groups had been unsuccessful in the thioester series, we investigated other potential protecting groups for primary amides. These were the phthalimide (**29**), 2,5-dimethyl pyrrole (**30**), hydrazide (**7n**), trityl (**7o**), dibenzyl (**7p**) and hexamethyldisilazane (**31**) substituted analogues (Figure 5).

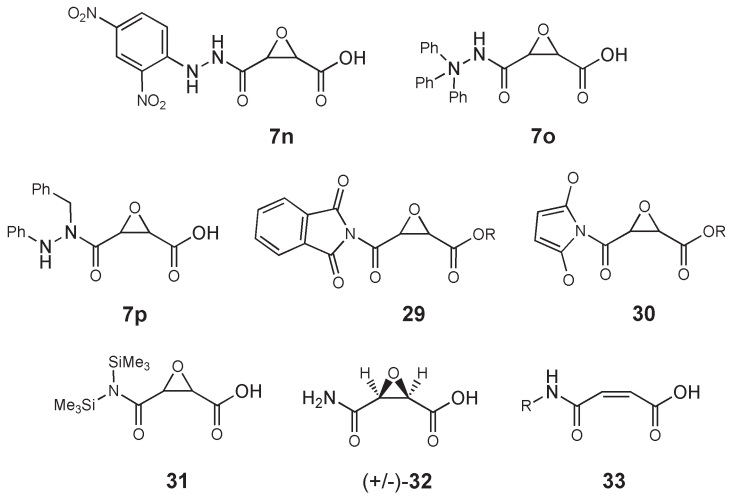


Figure 5

Reaction of anhydride **19** with potassium phthalimide under a variety of conditions failed to give the desired amide-acid (**29**, R = H). Reaction with ester-acid **4a** in place of the anhydride, also failed to give the desired product (**29**, R = Me), although some reaction did take place in this case (PCl_5, py.). A less common choice of primary amide protecting group is 2,5-dimethyl pyrrole [25] but again, reaction with either **19** or **4a** either with neat pyrrole or its sodium salt failed to give the desired amide acid/ester products (**30**, R = H or Me). In all cases, organic mass recoveries from the resulting black tars were low.

The hydrazido amide-acid (**7n**) and trityl amide-acid (**7o**) had been synthesized but as noted above their analogous amide-esters could not be prepared. Deprotection of the trityl group in **7o** might have hydrolysed the epoxide and/or ester as well, but hydrogenolysis of the N-N bond in **7n** would have been expected to be facile and would have given access to the ester-amide series **6**. Unfortunately, hydrogenation of **7n** was initially unsuccessful and not further investigated. Dibenzylamine **7p** could not be hydrogenated under standard conditions; more forcing conditions (40-50 psi H_2) might well have removed the benzyl group, but would almost certainly have reduced the epoxide group [26]. However, anhydride **19** successfully reacted with hexamethyldisilazane on refluxing in THF in high crude yield. Crystallization from methanol gave a yellow solid, which was not the expected silyl amide (**31**), but rather the primary amide acid (**32**) [27], an apparently novel compound [28].

A further effort to find a better route to the amide-acids **7** and amide-esters **8** was investigated by ring-opening maleic anhydride with amines in toluene to give the known crystalline maleamic-acids **33** in good yields [29]. However, no example of **33** could be epoxidised using either the classical H_2O_2/Na_2WO_4 catalysed conditions [19] or alkaline NaOCl, as reported by Tishler [10], presumably due to the strongly deactivated nature of the double bond. Finally, other oxidation states (protected alcohols and aldehydes) were investigated as alternative ways of arriving at the desired carboxylate functionalities, but these routes were potentially longer and less flexible, and the studies were inconclusive, and so are not reported here.

Conclusions.

In conclusion, we have synthesized a wide range of Cerulenin analogues (~40) covering all the structural subclasses of the proposed analogues, the majority of which constitute novel functional groupings. Our syntheses are short (four to five steps) and are from readily available starting materials. Most importantly, they are amenable to the addition of more complex side chains in a convergent fashion to the key head unit building blocks. These analogues have potential as inhibitors of both FAS and PKS multi-enzyme complexes. Biological results will be reported elsewhere.

EXPERIMENTAL

Melting points were determined on a Reichert hot-stage apparatus or a Buchi 510 melting point apparatus and are uncorrected. Elemental analyses (C, H, N and S) were performed by the "in house" chemistry department analytical service. Infra red spectra were run on Perkin-Elmer 297 or 1310 spectrophotometers between 4000 and 600 cm^{-1} and only readily assigned absorbances above ~1500 cm^{-1} are listed. ^1H NMR spectra were recorded on Bruker WM-250 (250 MHz), AC-250 (250 MHz), or AM-400 (400 MHz) spectrometers. The following additional abbreviations have been used for first order spectra: qu = quintet, sx = sextet, sp = septet, m = multiplet, b = broad. ^{13}C NMR spectra were recorded on Bruker AM-400 (100.6 MHz) or AC-250 (62.9 MHz) spectrometers and were proton decoupled. Electron impact (EI^+) mass spectra were recorded on Kratos MS 902 (ex A.E.I.) or MS 890 spectrometers. Fast atom bombardment (FAB^+) mass spectra (carrier gas Xe) were recorded on Kratos MS 50 or MS 890 spectrometers. Analytical TLC was carried out on commercially prepared plates coated with 0.25 mm of self-indicating Merck Kieselgel 60 F_{254} and were developed using a 5% solution of phosphomolybdic acid in ethanol. Preparative flash silica chromatography was performed using Merck Kieselgel 60 (230-400 mesh). All other reagents and solvents were purified and dried as necessary according to standard procedures.

cis-2,3-Epoxysuccinic anhydride (**19**) [20, 21].

The diacid **18** [19,20] (0.75 g, 5.7 mmol) was stirred overnight in TFAA (6 ml) at RT. The excess TFAA was removed by concentration and the pale pink residual solid was purified by sublimation at 50 °C and 0.1 mmHg for 3-4 hours to yield the title compound as colourless prisms (0.65 g, 100%); mp 64-65 °C (lit.[21] mp 63-64 °C); ir ($CHCl_3$): 1887, 1875, 1808 and 1756 cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 4.31 (s); ms: m/z (EI^+) 115 (M+1, 10%), 102 (35), 87 (25), 71 (95), 69 (M-CO_2H, 60), 59 (28), 44 (CO_2, 100). *Anal*. Calcd. for $C_4H_2O_4$: C, 42.12; H, 1.77. Found: C, 41.85; H, 1.74. Alternatively, the diacid **18** (0.99 g, 7.5 mmol) was heated at reflux with TFAA (2.1 ml, 15.0 mmol) in dichloromethane (8 ml) for 5 hours. The crude concentrate was purified by sublimation to yield the title compound as colourless prisms (0.79 g, 93%); mp 63-65 °C. Other data as above.

General Method for Epoxysuccinic Ester-Acids **4a-j**.

The anhydride **19** (114 mg, 1.0 mmol) was stirred the appropriate distilled alcohol (3-5 ml) for 3-48 hours. The excess alcohol was removed by evaporation at reduced pressure to give quantitative yields of the desired compounds which were used without further purification. Full characterisations are given only for those compounds isolated in their own right (*i.e.* the known **4a**, and **4h** and **4j**).

Monomethyl-*cis*-2,3-epoxysuccinate (**4a**) [21, 22].

This compound was isolated in quantitative yield as a viscous gum; ir ($CHCl_3$): 1760 (CO_2H), 1746 (CO_2Me) and 1711 (CO_2H) $cm^{-1;}$ ^1H NMR (250 MHz $CDCl_3$): δ 6.1-6.8 (1H, bs, OH), 3.85 (3H,s, Me), 3.80 (1H, d, *J*=4.9) and 3.77 (1H, d, *J*=4.9, epoxide); ms: m/z (EI^+) 147 (M+1, 2%), 129 (M-OH, 12), 115 (M-OMe, 10), 102 (M-CO_2, 27), 87 (20), 71 (100).

Monooctyl-*cis*-2,3-epoxysuccinate (**4h**).

This compound was isolated in quantitative yield as a white syrup; ir ($CHCl_3$): 2920, 2860 (C-H), 1750 (C=O) cm^{-1}; ^1H NMR (250 MHz, CDC_3): δ 7.5-8.2 (1H, bs, OH), 4.22 (2H, t, *J*=6.8, CH_2O), 3.78 (1H, d, *J*=4.7) and 3.74 (1H, d, *J*=4.7, epoxide), 1.66 (2H, qu, *J*=6.8, C*H*$_2$CH$_2$O), 1.27 (10H, m, $(CH_2)_5$), 0.87 (3H, t, *J*=6.5, CH_3); ms: m/z (EI^+) 244 (M^+, 100%), 199 (M-CO_2H, 45), 169 (85), 157 (55), 133 (100), 115 (100).

Anal. Calcd. for $C_{12}H_{20}O_5$: C, 58.99; H, 8.27. Found: C, 59.17; H, 8.24.

Monobenzyl-*cis*-2,3-epoxysuccinate (**4j**).

This compound was isolated in quantitative yield as a white waxy solid; mp 73-74 °C (neat); ir ($CHCl_3$): 3300-3600 (O-H), 2880, 2810 (C-H), 1757 (CO_2H), 1742 (CO_2Bz) cm^{-1}; ^1H NMR (250 MHz d_6-acetone): δ 7.35 (5H, m, PhO), 5.23 (1H, d, *J*=12.4) and 5.17 (1H, d, *J*=12.4, PhC*H*$_2$O), 3.93 (1H, d, *J*=4.7) and 3.85 (1H, d, *J*=4.7, epoxide); ms: m/z (EI^+) 222 (M^+, 25%), 178 (M-CO_2, 35), 150 (18), 115(70), 107 (PhCH_2O, 55), 91 (PhCH_2, 100).

Anal. Calcd. for $C_{11}H_{10}O_5$: C, 59.45; H, 4.55. Found: C, 59.25; H, 4.48.

General Method for Lower Homologue Symmetrical Epoxysuccinate Diesters **20b** and **5a**.

The following general method is exemplified by the procedure described below for the preparation of **20b**, starting from diacid **18** and other reagents scaled accordingly.

Diethyl-*cis*-2,3-epoxysuccinate (**20b**) [24].

Concentrated H_2SO_4 (7.8 g, 80 mmol) was added dropwise over 1 hour to a chilled suspension of the barium salt of **18** [19] (16.0 g, 60 mmol) in absolute ethanol (80 ml), keeping the temperature below 10 °C. The mixture was allowed to warm to 20 °C overnight and heated at reflux for 5 hours. On cooling, calcium carbonate (2.0 g) was added and the slurry filtered through Celite washing copiously with ether. The crude concentrate was partitioned between water (50 ml) and ether (50 ml), separated and the aqueous layer extracted with ether (3 x 40 ml). The combined ethereal extracts were washed sequentially with saturated $NaHCO_3$ solution (40 ml), water (40 ml), dried (Na_2SO_4) and concentrated to a yellow oil (6.1 g, 54%); ir ($CHCl_3$): 2900 (C-H), 1745 (C=O) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 4.18 (4H, q, *J*=7.1, 2 x CH_2O), 3.63 (2H, s, epoxide), 1.24 (6H, t, *J*=7.1, 2 x CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 165.62 (C=O), 61.85 (CH_2O), 52.41 (epoxide), 14.86 (CH_3); ms: m/z (EI^+) 188 (M^+, 40%), 161 (100), 143 (M-OEt, 100), 132 (30), 115 (100), 70 (100).

Dimethyl-*cis*-2,3-epoxysuccinate (**5a**) [22].

This compound was prepared according to the method for **20b** and isolated as a yellow oil (4.4 g, 56%); ir ($CHCl_3$): 1745 (C=O) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 3.79 (6H, s, 2, x Me), 3.71 (2H, s, epoxide); ms: m/z (EI^+) 160 (M^+, 75%), 147 (M-Me, 15), 129 (M-OMe, 100), 115 (80), 71 (80).

General Method for Higher Homologue Symmetrical Epoxysuccinate Diesters **20e** and **20k**.

The following general method is exemplified by the procedure described below for the preparation of **20e**, starting from diacid **18** and other reagents scaled accordingly.

Di-*tert*-butyl-*cis*-2,3-epoxysuccinate (**20e**).

PCl_5 (2.50 g, 12.0 mmol) was added on one portion to a chilled (0 °C) suspension of diacid **18** (0.66 g, 5.0 mmol) in dichloromethane (12 ml). The cooling bath was removed and solution stirred at 20 °C for 3 hours. The resulting homogeneous solution was cooled again to 0 °C and *t*-butyl alcohol (1.48 g, 20.0 mmol) dissolved in dichloromethane (1 ml) was added dropwise, followed by pyridine (2.1 ml, 30.0 mmol) after 5 minutes. The solution was warmed to 20 °C over 2 hours, diluted with chloroform (20 ml) and washed sequentially with 1 *M* H_2SO_4 (3 x 30 ml), saturated $NaHCO_3$ (2 x 30 ml), dried (Na_2SO_4), and concentrated to a brown oil. The crude product was purified by flash silica gel chromatography in $CHCl_3$ (R_f 0.28) to yield the title compound as a white solid (0.54 g, 44%); mp 101.5-102 °C ($CHCl_3$); ir ($CHCl_3$): 2900 (C-H), 1745 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 3.54 (2H, s, epoxide), 1.49 (9H, s, $(CH_3)_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 165.19 (C=O), 61.07, 52.89 (epoxide), 27.88 (CH_3); ms: m/z (EI^+) 244 (M^+, 22%), 187 (M-*t*Bu, 32), 171 (M-O *t*Bu, 100), 115 (100), 70 (75).

Anal. Calcd. for $C_{12}H_{20}O_5$: C, 58.99; H 8.27. Found: C, 58.84; H, 8.41.

Dibenzyl-*cis*-2,3-epoxysuccinate (**20j**).

This compound was isolated after flash silica gel chromatography in 4:1 hexane:ether (R_f 0.18) as a yellow waxy solid (89%); mp 45-48 °C (neat). ir ($CHCl_3$): 2890 (C-H), 1745 (C=O) cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$): δ 7.29-7.36 (10H, m, Ph), 5.07 (4H, s, 2 x CH_2O), 3.70 (2H, s, epoxide); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 165.45 (C=O), 134.71, 128.65, 128.61, 67.54 (CH_2O), 52.60 (epoxide); ms: m/z (EI^+) 312 (M^+, 55%), 223 (M-Bz, 17), 207 (M-BzO, 75), 107 ($PhCH_2O$, 45), 91 ($PhCH_2$, 100).

Anal. Calcd. for $C_{18}H_{16}O_5$: C, 69.21; H, 5.17. Found: C, 69.48; H, 5.15.

2-Chloro-1,4-dimethyl-3-hydroxysuccinate (**22**).

Thionyl chloride (3.7 ml, 51.0 mmol) was added dropwise to methanol (20 ml) cooled to –15 °C. Diacid **18** [19,20] (3.3g, 25.0 mmol) was added on one portion and the solution stirred at –10 °C for 1 hour before warming to 20 °C overnight. The crude concentrate was partitioned between saturated $NaHCO_3$ solution (30 ml) and chloroform (30 ml), separated and the aqueous layer extracted with chloroform (2 x 30 ml). The combined organic extracts were dried ($MgSO_4$) and concentrated to yield the title compound as a colourless oil (4.4 g, 86%); ir (film): 3500 (O-H), 2900 (C-H), 1753 (C=O) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 4.89 (1H, dd, *J*=7.5, 2.4, C*H*OH), 4.80 (1H, d, *J*=2.4, CHCl), 3.85 (3H, s, Me), 3.84 (3H, s Me), 3.31 (1H, d, *J*=7.5, OH); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.60, 166.80 (2 x C=O), 71.80 (CHOH), 59.66 (CHCl), 53.62 and 53.33 (2 x MeO); ms: m/z (FAB^+) 197/199 (M+1, 100), 137/139 (M-CO_2Me, 80).

Anal. Calcd. for $C_6H_9ClO_5$: C, 36.65; H, 4.62. Found: C, 36.90; H, 4.36.

General Method for Epoxysuccinamate Esters, **6c** and **6f-h**.

The following general method is exemplified by the procedure described below for the preparation of **6c**, using the appropriate crude ester-acid **4c** and other reagents scaled accordingly.

4-*iso*-Propyl-*cis*-2,3-epoxysuccinamate (**6c**).

PCl_5 (312 mg, 1.5 mmol) was added in one portion to a chilled (-5 °C) solution of crude ester-acid **4c** (220 mg, 1.25 mmol) in dichloromethane (6 ml). The ice-bath was removed and the solution allowed to warm to 20 °C over 3 hours. After this time the ice-bath was replaced and a dry ice-acetone condenser added. Liquid ammonia was condensed into the reaction mixture for 5-10 minutes, and then stirred for a further 30 minutes. After warming to 20°C, the reaction was quenched by the addition of water (3 ml), separated and extracted with chloroform (4 x 3 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated to a crude oil, which was purified by flash silica gel chromatography in 1:1 hexane:ethyl acetate (R_f 0.24) to yield the title compound as a white solid (118 mg, 55%); mp 136-138 °C; ir ($CHCl_3$): 3495, 3380 (N-H), 2920 (C-H), 1735 (CO_2R), 1685 (CONH) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 6.42 (1H, bs) and 5.66 (1H, bs, NH_2), 5.08 (1H, sp, *J*=6.3, C*H*$(CH_3)_2$), 3.68 (3H, d, *J*=5.0) and 3.65 (1H, d, *J*=5.0, epoxide), 1.29 (3H, d, *J*=6.3) and 1.24 (3H, d, *J*=6.3, CH(C*H*$_3)_2$); ms m/z (EI^+) 174 (M+1, 6%), 173 (M^+, 5), 158 (M-NH_2, 2), 132 (100), 130 (100), 114 (110), 88(100), 70(100).

Anal. Calcd. for $C_7H_{11}NO_4$: C, 48.54; H, 6.41; N, 8.09. Found: C, 48.55; H, 6.40; N, 8.16.

4-Hexyl-*cis*-2,3-epoxysuccinamate (**6f**).

This compound was isolated as a white waxy solid (64%); mp 136-137 °C; ir ($CHCl_3$): 3490, 3375 (N-H), 2905, 2840 (C-H), 1735 (CO_2R), 1680 (CONH) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 6.4 (1H, bs) and 5.6 (1H, bs NH_2), 4.18 (2H, t, *J*=6.8, CH_2O), 3.72 (1H, d, *J*=5.0) and 3.66 (1H, d, *J*=5.0, epoxide), 1.62 (2H, qu, *J*=7.0, C*H*$_2$CH$_2$O), 1.3 (6H, m, $(CH_2)_3$), 0.88 (3H, t, *J*=6.7, CH_3); ms: m/z (EI^+) 216 (M+1, 5%), 215 (M^+, 2), 199 (M-NH_2, 1), 172 (M-CONH, 3), 159 (6), 132 (100), 114 (100), 85 (100).

Anal. Calcd. for $C_{10}H_{17}NO_4$: C, 55.79; H, 7.98; N, 6.51. Found: C, 55.95; H, 7.93; N, 6.40.

4-Heptyl-*cis*-2,3-epoxysuccinamate (**6g**).

This compound was isolated as a white solid (67%); mp 42.5-43.5 °C; ir ($CHCl_3$): 3490, 3380m (N-H), 2910, 2850 (C-H), 1740 (CO_2R), 1685 (CONH) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 6.42 (1H, bs) and 5.57 (1H, bs, NH_2), 4.18 (2H, t, *J*=6.8, CH_2O), 3.72 (1H, d, *J*=5.0) and 3.66 (1H, d, *J*=5.0, epoxide), 1.61 (2H, m, C*H*$_2$CH$_2$O), 1.27 (8H, m, $(CH_2)_4$), 0.87 (3H, t, *J*=6.6, CH_3); ms: m/z (EI^+) 230 (M+1, 4%), 172 (2), 159 (3), 132 (100), 114 (100), 105 (28), 98 (40), 86 (97), 70 (99).

Anal. Calcd. for $C_{11}H_{19}NO_4$: C, 57.61; H, 8.37; N, 6.11. Found: C, 57.80; H, 8.32; N, 6.28.

4-Octyl-*cis*-2,3-epoxysuccinamate (**6h**).

This compound was isolated as a white solid (68%); mp 42-43 °C; ir ($CHCl_3$): 3495, 3380 (N-H), 2910, 2840 (C-H), 1740 (CO_2R), 1685 (CONH) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 6.42 (1H, bs) and 5.62 (1H, bs NH_2), 4.16 (2H, t, *J*=6.8, CH_2O), 3.72 (1H, d, *J*=5.0) and 3.66 (1H, d, *J*=5.0, epoxide), 1.64 (2H, qu, *J*=6.9, C*H*$_2$CH$_2$O), 1.25 (10H, m, $(CH_2)_5$), 0.87 (3H, t, *J*=6.6, CH_3); ms: m/z (EI^+) 244 (M+1, 15%), 200 (M-CONH, 5), 159 (12), 147 (86), 133 (95), 132 (100).

Anal. Calcd. for $C_{12}H_{21}NO_4$: C, 59.23; H, 8.72; N, 5.76. Found: C, 59.20; H, 8.97; N, 5.58.

General Method for Epoxysuccinamic Acids **7k-p**.

Anhydride **19** (generally 228 mg, 2.00 mmol) was heated at reflux in diethyl ether with the appropriate aniline (2.05 mmol) for 2-6 hours, or at reflux with the appropriate amine in THF for

18 hours. The title compounds were isolated by filtration from the cooled reaction mixture and recrystallised if necessary from acetone-hexane.

4-*N*-Phenyl-*cis*-2,3-epoxysuccinamic acid (**7k**) [20].

This compound was isolated as fine white needles (374 mg, 90%); mp 179-181°C (dec.) (lit.[20] 179-180 °C); ir (KBr): 3360 (N-H), 2500 (O-H), 1735 (C=O.O), 1640 (C=O.N), 1605, 1595 (C=C) cm^{-1}; ^1H NMR (80 MHz, d_6-acetone) 7.57-7.74 (2H, m) and 7.00-7.44 (3H, m, Ph), 3.94 (1H, d, *J*=5.0) and 3.87 (1H, d, *J*=5.0, epoxide); ms: m/z (EI^+) 207 (M^+, 60%), 163 (M-CO_2, 8), 119 (25), 105 (22), 93 ($PhNH_2$, 100), 77 (Ph^+, 40).

Anal. Calcd. for $C_{10}H_9NO_4$: C, 57.96; H, 4.39; N, 6.76. Found: C, 58.11; H, 4.36; N, 6.76.

4-*N*-(4-Methoxyphenyl)-*cis*-2,3-epoxysuccinamic acid (**7l**).

This compound was isolated as a purple coloured solid (391 mg, 82%); mp 160-162 °C (dec.) (acetone-hexane); ir (KBr): 3305 (N-H), 2510 (O-H), 1720 (C=O.O), 1640 (C=O.N), 1605 (C=C) cm^{-1}; ^1H NMR (250 MHz, d_6-acetone): δ 8.86 (1H, bs, NH), 7.57 (2H, dd) and 6.89 (2H, dd, *J*=6.9, 2.2, ArH), 3.90 (1H, d, *J*=5.0) and 3.86 (1H, d, *J*=5.0, epoxide), 3.77 (3H, s, MeO); ms: m/z (EI^+) 237 (M^+, 38%), 219 (M-H_2O, 3), 192 (M-CO_2H, 8), 148 (22), 133 (63), 121 ($MeOC_6H_4N^+$, 86), 107 ($MeOC_6H_4^+$, 72).

Anal. Calcd. for $C_{11}H_{11}NO_5$: C, 55.69; H, 4.68; N, 5.90. Found: C, 55.56; H, 4.68; N, 5.98.

4-*N*-(4-Nitrophenyl)-*cis*-2,3-epoxysuccinamic acid (**7m**) [20].

This compound was isolated as a light cream coloured solid (360 mg, 71%); mp 167-169 °C (dec.) (lit.[20] 178-179 °C); ir (KBr): 3355 (N-H), 2500 (O-H), 1730 (C=O.O), 1655 (C=O.N), 1615, 1595 (C=C) cm^{-1}; ^1H NMR (80 MHz, d_6-acetone): δ 9.5 (~1H, bs, OH), 8.25 (2H, dt, *J*=9.5, 2.4) and 7.95 (2H, dt, *J*=9.5, 2.4, ArH), 4.7-5.2 (~1H, vbs, NH), 4.00 (1H, d, *J*=5.0) and 3.93 (1H, d, *J*=5.0, epoxide); ms: m/z (EI^+) 252 (M^+, 100%), 208 (M-CO_2, 20), 138 (100), 115 (M-$ArNH^+$, 30).

Anal. Calcd. for $C_{10}H_8N_2O_6$: C, 47.62; H, 3.20; N, 11.11. Found: C, 47.41; H, 3.10; N, 11.23.

4-*N*-(2,4-Dinitrophenylhydrazino)-*cis*-2,3-epoxysuccinamic acid (**7n**).

This compound was isolated as a bright yellow solid (555 mg, 89%); mp 153-155 °C (dec.) (acetone-hexane); ir (KBr): 3340 (N-H), 2550 (O-H), 1720 (C=O.O), 1610, 1590 (C=O.N) cm^{-1}; ^1H NMR (250 MHz, $CDC1_3$): δ 9.6-11.5 (2H, vbs) and 9.80 (1H, bs, NHNH and OH), 8.98 (1H, d, *J*=2.6, Ar H-3), 8.28 (1H, dd, *J*=9.5, 2.6, Ar H-5), 7.56 (1H, d, *J*=9.5, Ar H-6), 4.01 (1H, d, *J*=4.9) and 3.95 (1H, d, *J*=4.9, epoxide); ms: m/z (FAB^+) 313 (M+1, 89%), 312(M^+, 36), 165 (16), 136 (92), 107 ($PhNHNH^+$, 33), 89 (34).

Anal. Calcd. for $C_{10}H_8N_4O_8$: C, 38.47; H, 2.59; N, 17.95. Found: C, 38.12; H, 2.65; N, 18.11.

4-*N*-Trityl-*cis*-2,3-epoxysuccinamic acid (**7o**).

This compound was isolated as a white solid (255 mg, 67%); mp 175-179 °C (dec.) (acetone-hexane); ir (KBr): 2500 (O-H), 1730 (C=O.O), 1630 (C=O.N); ^1H NMR (250 MHz, d_6-acetone): δ 7.89 (1H, s, NH), 7.20-7.39 (15H, m, Ph_3CN), 3.94 (1H, d, *J*=5.1) and 3.73 (1H, d, *J*=5.1, epoxide); ms: m/z (EI^+) 374 (M+1, 75%), 356 (M-OH, 44), 340 (63), 311 (100), 243 (100), 192 (100), 104 (100).

Anal. Calcd. for $C_{23}H_{19}NO_4$: C, 73.97; H, 5.14; N, 3.75. Found: C, 73.57; H, 5.17; N, 3.83.

4-(*N,N*-Dibenzyl)-*cis*-2,3-epoxysuccinamic acid (**7p**).

This compound was isolated as white prisms (448 mg, 72%); mp 147-148 °C (acetone-hexane); ir (KBr): 2500 (O-H), 1730 (C=O.O), 1620 (C=O.N); ^1H NMR (250 MHz, d_6-acetone): δ 7.24-7.39 (10H, m, Ph_2N), 4.77 (1H, d, *J*=16.4) and 4.65 (1H, d, *J*=4.7, $PhCH_2N$), 4.63 (1H, d, *J*=14.9) and 4.39 (1H, d, *J*=14.9, $Ph'CH_2N$), 4.15 (1H, d, *J*= 4.7) and 3.83 (1H, d, *J*=4.7, epoxide), 3.77 (3H, s, MeO); ^{13}C NMR (100.6 MHz, d_6-acetone): δ 168.38 and 166.31 (2 x C=O), 137.53, 137.34, 129.50, 129.25, 128.94, 128.41 and 128.06 (Ph_2N), 55.04 and 53.85 (epoxide), 50.00 and 48.14 (CH_2NCH_2); ms: m/z (FAB^+) 312 (*M*+1, 69%), 289 (8), 198 (Ph_2NH_2, 46), 154 (78), 136 (69), 107 ($PhCH_2NH_2$, 26), 91 ($PhCH_2^+$, 100).

Anal. Calcd. for $C_{18}H_{17}NO_4$: C, 69.43; H, 5.51; N, 4.50. Found: C, 69.46; H, 5.53; N, 4.58.

4-*N*-(4-Methoxybenzyl)-*cis*-2,3-epoxysuccinamide (**9q**).

DCC (413 mg, 2.00 mmol) and DMAP (12 mg, 0.10 mmol) were added to a solution of ester-acid **4a** (146 mg, 1.00 mmol) in dichloromethane (5ml) cooled to -5°C. After stirring for 5 minutes, 4-methoxybenzylamine (143 µL, 1.10 mmol) was added by syringe in one portion and the reaction mixture stirrred at 20 °C for 24 hours. The solution was filtered through a pad of Celite, washed with dichloromethane and concentrated to dryness (600 mg). The crude concentrate was purified by flash silica gel chromatography in 1:1 hexane:ether (R_f 0.28) to yield the semi-pure **8q** as a buff solid (100 mg, 38%) which was used without further purification. The amide-ester **8q** (59 mg, 0.22 mmol) was stirred with 0.88 aqueous ammonia solution (40 µl, 0.80 mmol) and methanol (1.0 ml) in a sealed flask for 5 days. The resulting precipitate was collected by filtration and dried *in vacuo* on an oil pump to yield the title compound as a buff coloured solid (24 mg, 43%); mp 177-178 °C; ir (KBr): 3460 (N-H), 3290, 3180 (NH_2), 1670, 1645 (CONH and $CONH_2$) cm^{-1}; ^1H NMR (250 MHz, d_6-DMSO): δ 8.37 (1H, bt *J*=6.0, ArNHCO), 7.52 (1H, bs) and 7.37 (1H, bs, $CONH_2$), 7.17 (2H, d, *J*=8.6) and 6.86 (2H, d, *J*=8.7, Ar-H), 4.28 (1H, dd, *J*=14.9, 6.2) and 4.13 (1H, dd, *J*=14.9, 5.8, $ArCH_2N$), 3.73 (3H, s, MeO), 3.72 (1H, d, *J*=5.3) and 3.63 (1H, d, *J*=5.2, epoxide); ^{13}C NMR (100.6 MHz, d_6-DMSO): δ 167.33 and 165.13 (2 x C=O), 158.35, 130.73, 128.75 and 113.78 (C=C), 55.18 (MeO), 54.47 and 54.41 (epoxide), 41.53 ($ArCH_2N$); ms: m/z (EI^+) 206 (M-$CONH_2$, 72%), 163 (44), 136 ($ArCH_2NH^+$, 100), 121 ($ArCH_2^+$, 77).

Anal. Calcd. for $C_{12}H_{14}N_2O_4$: C, 57.58; H, 5.65; N, 11.20. Found: C, 57.20; H, 5.77: N, 10.93.

1-Ethyl-4-*N*-(4-methoxybenzyl)-*cis*-2,3-epoxysuccinamate (**24**).

The method of **6c** was used starting from crude ester-acid **4b** and 4-methoxybenzylamine to yield the title compound after flash silica gel chromatography in 2% methanol:dichloromethane (R_f 0.48) as a white solid (376 mg, 67%); mp 102.5-103 °C; ir ($CHCl_3$): 3395 (N-H), 1738 (C=O.O), 1661 (CONH), 1605 (C=C) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 7.19 (2H, d, *J*=8.6) and 6.84 (2H, d, *J*=8.6, Ar-H), 6.65-6.75 (1H, bs, NH), 4.39 (1H, dd, *J*=14.5, 6.2) and 4.29 (1H, dd, *J*=14.5, 5.6, $ArCH_2N$), 4.12 (2H, q, *J*=7.1, OCH_2), 3.78 (3H, s, OMe), 3.71 (1H, d, *J*=5.0) and 3.68(1H, d, *J*=5.0, epoxide), 1.22 (3H, t, *J*=7.1, CH_2CH_3); ms: m/z (EI^+) 279 (M^+, 100%), 206 (M-CO_2Et, 100), 177 (80), 164 (50), 136 ($ArCH_2NH^+$, 100), 121 ($ArCH_2^+$, 100), 109 (60).

Anal. Calcd. for $C_{14}H_{17}NO_5$: C, 60.20; H, 6.15; N, 5.02. Found: C, 60.05; H, 6.09; N, 4.93.

General Method for Epoxysuccinic Thioester-Esters, **11**, **25**, **26**, and **28**.

The following general method is exemplified by the procedure described below for the preparation of **11c**, using the appropriate ester-acid **4a** and other reagents scaled accordingly.

4-Methyl-1-*S*-(*iso*-propyl)-*cis*-2,3-epoxysuccin-1-thioate (**11c**).

DCC (413 mg, 2.00 mmol) and DMAP (12 mg, 0.10 mmol) were added to a solution of ester-acid **4a** (146 mg, 1.00 mmol) in THF (5 ml) cooled to –5 °C. After stirring for 5 minutes, *iso*-propane thiol (93 µL, 1.0 mmol) was added by syringe in one portion and stirring continued at this temperature for 45 minutes or until TLC indicated the reaction was completed. The crude reaction mixture was concentrated to dryness and purified by flash silica gel chromatography in 4:1 hexane:ether (R_f 0.17) to yield the title compound as a yellow oil (90 mg, 44%); ir ($CHCl_3$): 2855 (C-H), 1748 (C=O.O), 1665 (C=O.S) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 3.76 (3H, s, OMe), 3.76 (1H, d, *J*=4.8) and 3.72 (1H, d, *J*=4.8, epoxide), 3.71 (1H, sp, *J*=6.9, C*H*(CH_3)$_2$), 1.29 (6H, d, *J*=6.9, CH (C*H*$_3$)$_2$); ^{13}C NMR (100.6 MHz; $CDCl_3$): δ 193.13 (C=O.S), 165.74 (C=O.O), 58.01 (C-2), 53.67 (C-3), 52.63 (MeO), 35.01 (SCH), 22.63 and 22.51 (CH(*C*H$_3$)$_2$); ms: m/z (EI$^+$) 204 (M$^+$, 48%), 173 (M-MeO, 31) 162 (M-Me$_2$C$^+$, 18), 129 (M-S*i*Pr, 100), 101 (M-COS*i*Pr, 94).

Anal. Calcd. for $C_8H_{12}O_4S$: C, 47.04; H, 5.93; S 15.70. Found: C, 47.13; H, 5.71; S, 15.51.

1-*S*-(*tert*-Butyl)-4-methyl-*cis*-2,3-epoxysuccin-1-thioate (**11e**).

This compound was isolated as a yellow oil (56%); ir ($CHCl_3$): 2900, 2855 (C-H), 1726 (C=O.O), 1658 (C=O.S) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 3.76 (3H, s OMe), 3.69 (2H, s, epoxide), 1.46 (9H, s, C(CH_3)$_3$); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 193.02 (C=O.S), 165.89 (C=O.O), 57.96 (C-2), 53.50 (C-3), 52.57 (MeO), 49.30 (*C*(CH_3)$_3$), 29.63 (C(CH_3)$_3$); ms: m/z (FAB$^+$) 219 (M+1, 73%), 185 (12), 163 (100).

Anal. Calcd. for $C_{19}H_{14}O_4S$: C, 49.52; H, 6.48; S, 14.69. Found: C, 49.59; H, 6.39; S, 14.86.

1-*S*-Heptyl-4-methyl-*cis*-2,3-epoxysuccin-1-thioate (**11g**).

This compound was isolated as a yellow oil (79%); ir ($CHCl_3$): 2910, 2850 (C-H), 1750 (C=O.O), 1670 (C=O.S) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 3.79 (1H, d, *J*=4.7) and 3.74 (1H, d, *J*=4.7, epoxide), 3.77 (3H, s, OMe), 2.92 (2H, t, *J*=7.3, CH_2S), 1.56 (2H, qu, *J*=7.0, C*H*$_2$CH$_2$S), 1.26 (8H, m, (CH_2)$_4$), 0.86 (3H, t, *J*=6.4, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): 193.25 (C=O.S), 165.67 (C=O.O), 58.13 (C-2), 53.71 (C-3), 52.63 (MeO), 31.60, 29.12, 28.74, 28.68, 28.63, 27.74 and 22.52 (7 x CH_2), 14.00 (CH_3); ms: m/z (EI$^+$) 261 (M+1, 10%), 260 (M$^+$, 4), 229 (M-MeO, 85), 201 (M-MeO$_2$C,49), 159 (100), 129 (100), 101 (100).

Anal. Calcd. for $C_{12}H_{20}O_4S$: C, 55.35; H, 7.76; S, 12.31. Found: C, 55.58; H, 7.87; S, 12.45.

4-Methyl-1-*S*-pentyl-*cis*-2,3-epoxysuccin-1-thioate (**11r**).

This compound was isolated as a yellow oil (75%); ir ($CHCl_3$): 2905, 2840 (C-H), 1735 (C=O.O), 1660 (C=O.S) cm^{-1}; ^1H NMR (250MHz, $CDCl_3$): δ 3.79 (1H, d, *J*=4.7) and 3.73 (1H, d, *J*=4.7, epoxide), 3.76 (3H, s, OMe), 2.91 (2H, t, *J*=7.2, CH_2S), 1.56 (2H, qu, *J*=7.1), 1.33 (4H, m, CH_2CH_2), 0.87 (3H, t, *J*=7.3, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 193.30 (C=O.S), 165.70 (C=O.O), 58.14 (C-2), 53.72 (C-3), 52.68 (MeO), 30.79, 28.80, 28.71 and 22.11 (4x CH_2), 13.87 (CH_3); ms: m/z (FAB$^+$) 233 (M+1, 100%), 231 (M-1, 14), 201 (M-MeO, 28).

Anal. Calcd. for $C_{10}H_{16}O_4S$: C, 51.70; H, 6.96; S, 13.80. Found: C, 51.86; H, 6.86; S, 13.64.

4-Butyl-1-*S*-heptyl-*cis*-2,3-epoxysuccin-1-thioate (**25t**).

This compound was isolated as a yellow oil (32%); ir (CHC_3): 2920, 2860 (C-H), 1745 (C=O.O), 1670 (C=O.S) cm^{-1}; ^1H NMR (250 MHz, $CDCl_3$): δ 4.16 (2H, q, *J*=6.7, OCH_2), 3.78 (1H, d, *J*=4.7) and 3.71 (1H, d, *J*=4.7, epoxide), 2.91 (2H, t *J*=7.3, CH_2S), 1.50-1.67 (4H, m, C*H*$_2$CH$_2$O and C*H*$_2$CH$_2$S), 1.26-1.43 (10H, m, (CH_2)$_5$), 0.91 (3H, t, *J*=7.2, CH_3), 0.86 (3H, t, *J*=6.7, CH_3); ms: m/z (EI$^+$) 303 (M+1, 19%), 242 (27), 229 (M-nBuO, 44), 201 (M-*n*BuOCO, 42), 183 (25), 171 (M-HepS, 100), 159 (100), 131 (HepS$^+$, 100), 115 (100).

Anal. Calcd. for $C_{15}H_{26}O_4S$: C, 59.56; H, 8.68; S, 10.60. Found: C, 59.73; H, 8.69; S, 10.77.

4-Benzyl-1-*S*-pentyl-*cis*-2,3-epoxysuccin-1-thioate (**25u**).

This compound was isolated as a yellow oil (58%); ir ($CHCl_3$): 2932, 2860 (C-H), 1757 (C=O.O), 1672 (C=O.S) cm^{-1}; ^1H NMR (250MHz, $CDCl_3$): δ 7.34 (5H, m, Ph), 5.19 (2H, s, PhC*H*$_2$), 3.80 (1H, d, *J*=4.7) and 3.76 (1H, d, *J*=4.7, epoxide), 2.83 (2H, m, CH_2S), 1.52 (2H, qu, *J*=7.2, C*H*$_2$CH$_2$S), 1.31 (2H, m, C*H*$_2$CH$_3$), 0.87 (3H, t, *J*=6.9, CH_3); ms: m/z (EI$^+$) 308 (M$^+$, 23%), 217 (M-Bz, 51), 202 (M-PhCHO$^+$, 100), 194 (100), 173 (M-BzO$_2$C, 100), 131 (*n*PenSCO, 84), 115 (100), 107 (BzO, 100), 103 (100), 91 (Bz$^+$, 100).

Anal. Calcd. for $C_{16}H_{20}O_4S$: C, 62.30; H, 6.55; S, 10.39. Found: C, 62.45; H, 6.68; S, 10.57.

1,4-Dipentyl-*cis*-2,3-epoxysuccin-1,4-dithioate (**26**).

This compound was prepared according to the method of **11c** starting from diacid **18** and pentane thiol to yield the title compound after purification by flash silica gel chromatography in 14:1 hexane:ether (R_f 0.21) as a yellow oil (75%); ir ($CHCl_3$): 2900, 2840 (C-H), 1658 (C=O.S) cm^{-1}; ^1H NMR (250MHz, $CDCl_3$): δ 3.84 (2H, s, epoxide), 2.92 (4H, t, *J*=7.3, 2 x CH_2S), 1.55 (4H, m, 2 x C*H*$_2$CH$_2$S), 1.33 (8H, m, 2 x (CH_2)$_2$), 0.87 (3H, t, *J*=7.0, 2 x CH_3); ms: m/z (EI$^+$) 304 (M$^+$, 45%), 303 (M-1, 15), 201 (M-S*n*Pen, 100), 71 (*n*Pen$^+$, 57).

Anal. Calcd. for $C_{16}H_{20}O_4S$: C, 55.22; H, 7.96; S, 21.06. Found: C, 55.56; H, 7.92; S, not determined as high S content outside range of method used.

1-Benzyl-4-butyl-*cis*-2,3-epoxysuccinate (**28**).

This compound was prepared according to the method of **11c** starting from ester-acid **4j** and *n*butanol to yield the title compound after purification by flash silica gel chromatography in 14:1 hexane:ether (R_f 0.21) as a clear oil (62%); ir ($CHCl_3$): 2945, 2882 (C-H), 1750 (C=O.O) cm^{-1}; ^1H NMR (250MHz, $CDCl_3$): δ 7.35 (5H, m, Ph), 5.20 (2H, s, PhC*H*$_2$O), 4.06 (3H, t, *J*=6.8, CH_2O), 3.72 (1H, d, *J*=4.6) and 3.68 (1H, d, *J*=4.6, epoxide), 1.54 (2H, m, C*H*$_2$CH$_2$O), 1.32 (2H, sx, *J*=7.4, C*H*$_2$CH$_3$), 0.90 (3H, t, *J*=7.2, CH_3); ^{13}C NMR (100.6 MHz, $CDCl_3$): 165.65 and 165.56 (2 x C=O.O), 134.70, 128.66 and 128.63 (Ph), 67.62 and 65.87 (2 x CH_2O), 52.67 and 52.55 (epoxide), 30.30, 18.89, 13.61; ms: m/z (EI$^+$) 278 (M$^+$, 100%), 221 (M-Bu$^+$, 83), 180 (100), 177 (M-BuO$_2$C$^+$, 100), 172 (M-PhCHO$^+$, 100), 107 (PhCH$_2$O, 100), 91 (PhCH$_2^+$, 100).

Anal. Calcd. for $C_{15}H_{18}O_5$: C, 64.73; H, 6.53. Found: C, 64.64; H, 6.46.

Hydrogenation of **28** to give **4d**.

Benzyl ester (**28**) (72 mg, 0.26 mmol) was stirred with 10% Pd/C (10 mg) in THF (2.0 ml) under an atmosphere of H_2 for 75 mins. The reaction mixture was filtered through a pad of Celite, washed copiously with ether and concentrated to dryness to yield crude **4d** as a clear oil (41 mg, 81%).

Unsuccessful Hydrogenations of **25u** to give **10r**.

Benzyl thioester (**25u**) (81 mg, 0.26 mmol) was stirred with 10% Pd/C (10 mg) in ether (3.0 ml) under an atmosphere of H_2 for 2.5 hours, after which time, TLC indicated no reaction had occurred. Further catalyst (70 mg) was added and the reaction continued for 20 hours. Still no reaction occurred so the recovered **25u** (80 mg) was heated to reflux in fresh ether with a large excess of wood charcoal for 1 hour, before being filtered and concentrated to dryness. The concentrate was dissolved in THF (5.0 ml) and stirred under an atmosphere of H_2 with 10% Pd/C (120 mg) for 3 days. TLC and ^{1}H NMR analysis indicated no reaction and **25u** was recovered quantitatively. Lastly, **25u** (40 mg, 0.13 mmol) was stirred with Pearlman's catalyst ($Pd(OH_2)$/C) (9 mg) in ethanol (2.0 ml) under an atmosphere of H_2 for 6 days. TLC and ^{1}H NMR analysis again indicated no reaction and **25u** was recovered quantitatively.

cis-2,3-Epoxysuccinamic acid (**32**).

Anhydride **19** (228 mg, 2.0 mmol) and hexamethyldisilazane (420 µl, 2.0 mmol) were stirred in ether (3.5 ml) for 4 days at RT (or alternatively heated at reflux in THF for 6 hours). The solution was concentrated to dryness to yield the crude title compound as an orange gum that solidified on standing. One crystallisation from methanol gave a pale yellow solid (164 mg, 63%); mp 158-160 °C (dec.); ir (KBr): 3435, 3200 (NH_2), 2500-3150 (O-H), 1735 (C=O.O), 1680, 1630 (C=O.N) cm^{-1}; ^{1}H NMR (250MHz, d_6-DMSO): δ 13.3 (1H, vbs, OH), 7.52 (1H, bs) and 7.26 (1H, bs, NH_2), 3.75 (1H, d, *J*=5.1) and 3.63 (1H, d, *J*=5.1, epoxide); ^{13}C NMR (100.6 MHz, d_6-DMSO): δ 167.75 and 167.28 (C=O.OH and C=O.NH), 53.63 and 52.81 (epoxide); ms: m/z (CI^-) (%) 130 (M-1, 100%), 113 (M-OH, 88), 112 (M-H_2O, 64), 86 (14), 81 (38), 79 (39).

Anal. Calcd. for $C_4H_5NO_4$: C, 36.64; H, 3.85; N, 10.69. Found: C, 36.26; H, 3.71; N, 10.60.

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