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Bioorganic & Medicinal Chemistry 15 (2007) 142–159
Bioorganic & Medicinal Chemistry

Novel selective inhibitors of neutral endopeptidase for the treatment of female sexual arousal disorder

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Received 22 May 2006; revised 27 September 2006; accepted 4 October 2006
Available online 6 October 2006

Abstract—A series of substituted glutaramides were synthesised using Candoxatrilat **1** as a lead and evaluated for potency against neutral endopeptidase (NEP) as a potential treatment for female sexual arousal disorder (FSAD). In this paper, we describe studies in which we were able to increase NEP activity substantially over the levels reported for previous compounds from this programme by appropriate substitution in both the P_1' and P_2' regions. Optimisation led to the 4-chlorophenpropylamide ***S*-30** which was selected as a candidate for further study.

1. Introduction

Female sexual arousal disorder (FSAD)[†] is believed to be a highly prevalent sexual disorder affecting significant numbers of pre-, peri-, and post-menopausal women.[1] It has been suggested that the most common cause of FSAD is decreased genital blood flow, resulting in reduced vaginal, labial and clitoral engorgement.[2] In the previous papers in this series,[3] we introduced a series of mono-carboxylic acid glutaramide inhibitors of the Zn-dependent metallopeptidase Neutral Endopeptidase [NEP, EC (3.4.24.11)] which we targeted as potential therapeutic agents for the treatment of FSAD through the potentiation of vasoactive intestinal peptide (VIP)-induced increases in genital blood flow. These acids were based upon the Candoxatrilat (**1**) template,[4] wherein by targeting small (Mwt < 400), relatively polar ($0 < \log D < 1$) mono-carboxylic acid structures we were able to identify several compounds which were potent ($IC_{50} < 50$ nM), selective for NEP over related peptidases and demonstrated low clearance (Cl) and volume of distribution (V_d) in animal models. Most notably, the amino-thiadiazole ***R*-2** was shown to possess the best combination of potency and a pharmacokinetic profile suitable for *prn* or on-demand dosing (short elimination $T_{1/2}$, rapid onset of action), and was selected as the prototype clinical candidate from the programme (Fig. 1).[5]

In this paper, we describe further explorations of the SAR of this series of small glutaramides using this strategy, within which we sought a back-up agent to ***R*-2** which was differentiated by a significant, ideally >10-fold increase in NEP activity by appropriate substitution in both the P_1' and P_2' regions while largely retaining the basic Candoxatrilat template. The SAR optimisation which led to the 4-chlorophenpropylamide (***S*-30**) is described below.[6]

Keywords: Neutral endopeptidase; NEP; Glutaramides; FSAD.
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† *Abbreviations used:* FSD, female sexual dysfunction; FSAD, female sexual arousal disorder; VIP, vasoactive intestinal peptide; NEP, neutral endopeptidase; ACE, angiotensin converting enzyme; ECE, endothelin converting enzyzme; WSCDI, (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimde); DEAD, 1,1-diethylazodicarboxylate; DPPA, diphenylphosphoryl azide; LDA, lithium diisopropylamide; HOBt, 1-hydroxybenzotriazole; *n*-Hept, *n*-heptyl; *n*-Pent, *n*-pentyl; NMM, *N*-methylmorpholine.

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doi:10.1016/j.bmc.2006.10.002

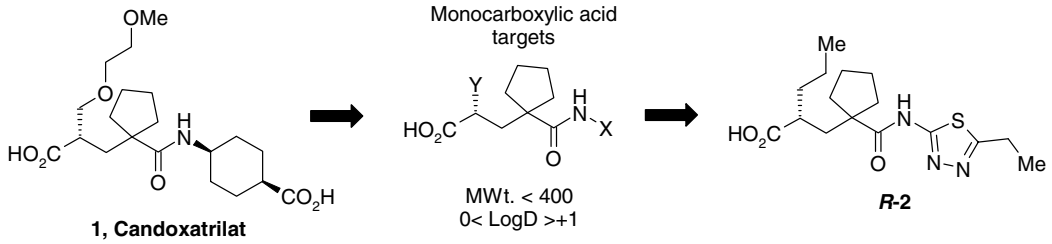
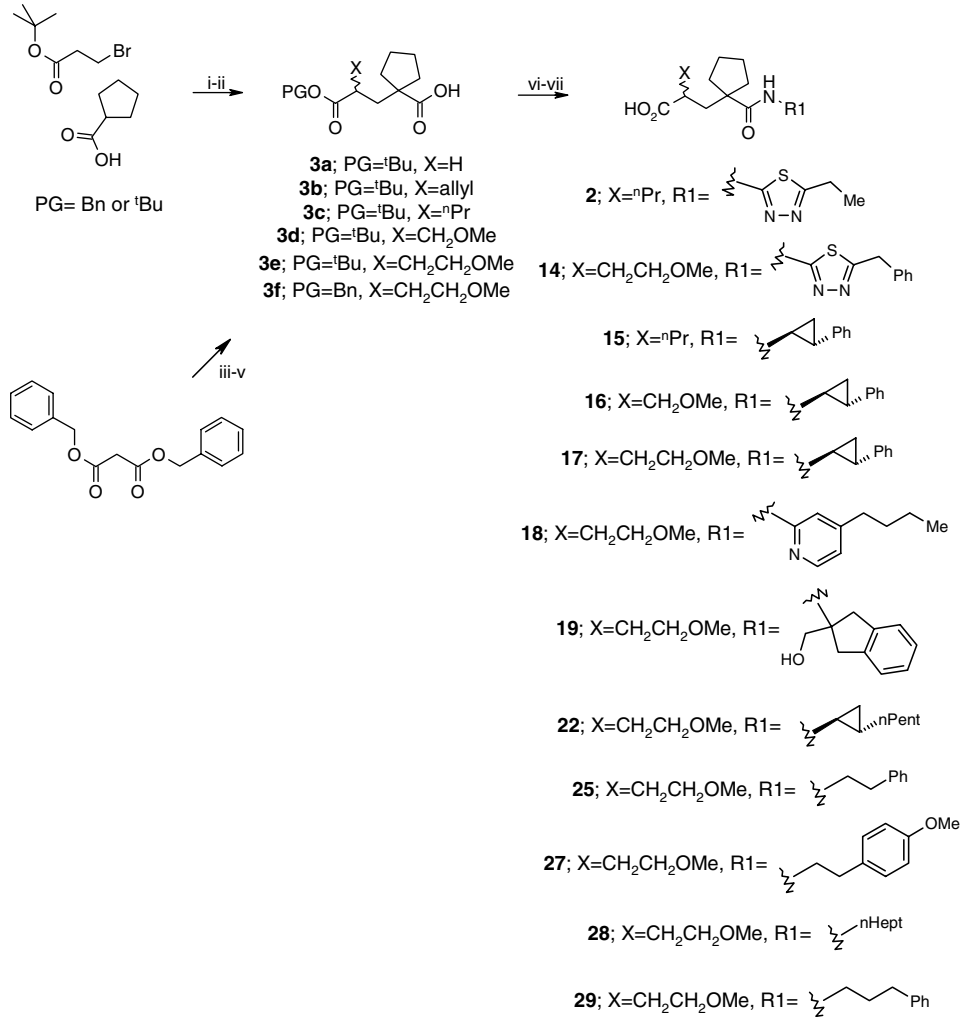


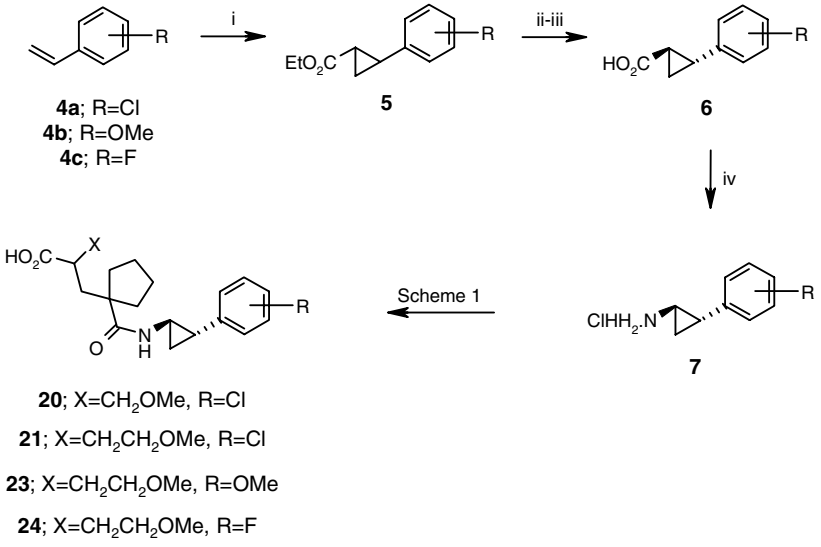
Figure 1. Lead structures and medicinal chemistry strategy.

2. Synthetic chemistry

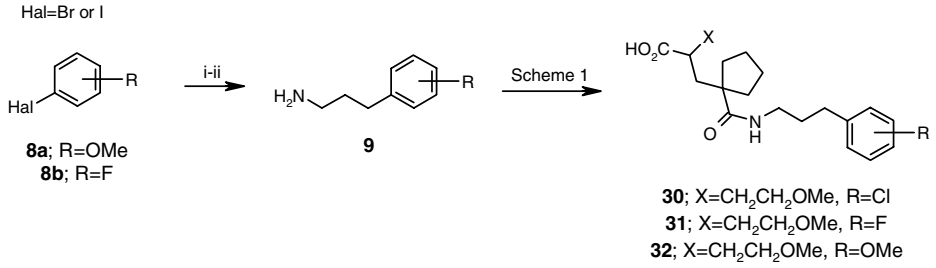
The glutaric acid template **3** is easily constructed by alkylation of cyclopentane carboxylic acid dianion (Scheme 1).[3] Coupling of commercially available and custom-synthesised amines to this acid was then performed using WSCDI with NMM as a base. For reactive amines (e.g., primary alkyl amines) DCM as solvent at room temperature was sufficient to achieve complete reaction. For less active amines (e.g., amino-substituted heterocycles) DMF or acetonitrile as solvents at elevated temperatures were required for good yields of the coupled products. The ester protecting group could then be removed using standard protocols to deliver the requisite acids in excellent yields.



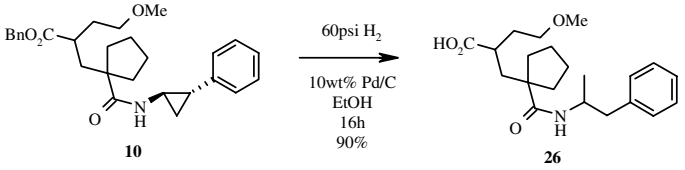
Scheme 1. Reagents and conditions: (i) **3a**; 2 equiv LDA, THF, −20 °C → room temperature, 16 h, 29%; (ii) **3b**; 2 equiv LDA, allyl bromide, THF, −78 °C → room temperature, 16 h, 92%; **3c**; then 15 psi H_2, EtOH, 10% Pd/C, room temperature, 1 h, 91% OR **3e**; 2 equiv LDA, bromooethylmethyl ether, THF, −78 °C → room temperature, 16 h, 55% OR **3d**; 2 equiv LDA, chloromethyl methyl ether, THF, −78 °C → room temperature, 16 h, 66%; (iii) bromoethylmethyl ether, NaH, THF, room temperature → reflux, 16 h, 70%; (iv) KOH, water, dioxan, rt, 16 h, then $(HCHO)_n$, piperidine, pyridine, 60 °C, 2.5 h, 46%; (v) cyclopentane carboxylic acid, 2 equiv LDA, THF −78 °C → room temperature, 5 h, 34%; (vi) $R1NH_2$, WSCDI, HOBt·NMM, solvent (see text), 25–100 °C, 12 h, 38–92%; (vii) TFA/DCM (1:1), room temperature, 6–16 h or HCl (g), Et_2O, DCM or EtOAc, room temperature, 10 min, 12–97%.



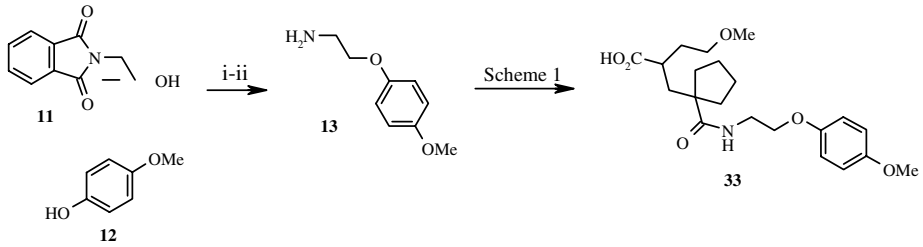
Scheme 2. Reagents and conditions: (i) EtO_2CCHN_2, $Rh_2(OAc)_4$, toluene, 1 h, 85 °C, 31–41%; (ii) NaOMe, EtOH, 85 °C, 18 h; (iii) LiOH (aq), MeOH, 70 °C, 16 h, 78–96% over 2 steps; (iv) DPPA, NEt_3, ^{t}BuOH, 90 °C, 48 h, 80–89%.



Scheme 3. Reagents and conditions: (i) acrylonitrile, P(*o*-Tol)$_3$, Pd (OAc)$_2$, NEt_3, MeCN, 80 °C, 14 h, 45–56%; (ii) NH_3 (aq), EtOH, 40 psi H_2, Ra–Ni, 25 °C, 12 h, 43–88%.



Scheme 4.



Scheme 5. Reagents and conditions: (i) DEAD, PPh_3, DCM, 25 °C, 16 h, 50%; (ii) hydrazine hydrate, methanol, reflux, 4 h then 25 °C, 48 h, 63%.

Chiral, non-racemic versions of the acids **3** were obtained from the racemic material using a (+)-pseudoephedrine-mediated classical resolution procedure[7] described in Section 5. Non-commercial and novel amines were prepared by the procedures described in Schemes 2–5. Thus, 1-amino-2-aryl cyclopropanes **7**

were prepared by an initial cyclopropanation catalysed by rhodium acetate dimer, and then equilibrated and hydrolysed to the most thermodynamically stable *trans*-1-carboxyl-2-aryl cyclopropanes **6**. A Curtius rearrangement provided the aminocyclopropanes **7**, which were then coupled to the acids **3** according to the procedure of Scheme 1.

Several phenpropylamines were prepared by Heck reaction (Scheme 3) of the corresponding aryl halides **8** with acrylonitrile, followed by reduction with Raney nickel to the requisite amines **9** and coupling to the acids **3**. In the case of *p*-chloroiodobenzene, the reduction step was accompanied by some dechlorination, and so an alternative literature method of preparing this example of amine **9** was used.[8]

The α-methyl phenethylamine **26** was made by simple hydrogenation of the benzyl ester analogue **10**.

The phenoxyethylamine **33** was prepared by the 4 step sequence outlined in Scheme 5. *N*-hydroxyethyl phthalimide **11** underwent a smooth Mitsunobu reaction with 4-methoxy phenol **12**. Deprotection and coupling according to Scheme 1 provided the phenoxy compound **33** in good yield after deprotection.

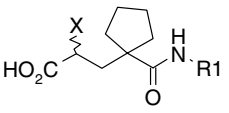
3. Results and discussion

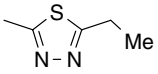
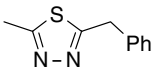
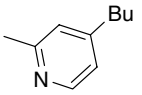
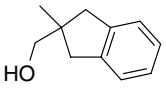
Compounds were first evaluated in vitro as inhibitors of canine NEP (dNEP) using a spectrophotometric assay, involving the cleavage of Abz-D-Arg-Arg-Leu-EDDnp to generate the fluorescent product Abz-D-Arg-Arg.[9] Soluble NEP was obtained from the kidney cortex by an adaptation of a literature method.[10] Selectivity over the related peptidases porcine angiotensin converting enzyme (pACE) and human endothelin converting enzyme (hECE-1) was established using Abz-Gly-*p*-nitro-Phe-Pro-OH and Big ET-1 as substrates, respectively. Potent and selective compounds were assayed against other NEP species, including human NEP (hNEP), obtained from human kidney, and progressed into in vitro metabolic stability assessment (including log *D* and Caco-2 cell permeability measurement) and in vivo pharmacokinetic evaluation; promising compounds which emerged from this analysis were assessed for efficacy in an animal model of genital blood flow[11] the results of which will be described elsewhere.

3.1. Amide SAR

The racemic version of the ethyl aminothiadiazole **2** is a potent and selective inhibitor of dog NEP with an IC_{50} of 60 nM (Table 1).[3] Increasing the size of the ethyl substituent to a benzyl group was proposed to increase potency through making additional hydrophobic contacts, albeit with an incumbent increase in lipophilicity. Therefore, an oxygen atom was also inserted into the P_1' propyl side chain to modulate the log *D* of this target. Compound **14** did indeed prove to be some 10-fold more potent than **2**. The

Table 1. Dog NEP activity for various substituted glutaramides



Compound	X	R^1	dNEP IC_{50} (nM)
2	^{n}Propyl		60
14	Methoxyethyl		5
15	^{n}Propyl		160
16	Methoxyethyl		100
17	Methoxymethyl		65
18	Methoxyethyl		17
19	Methoxyethyl		11

[a]Mean data of at least two determinations.
[b]All entries are racemic mixtures.
[c]All compounds showed >3 μM activity versus pACE.

effects of these modifications were further investigated through retaining a phenyl-containing P_2' group, but this time using a cyclopropane ring as an alternative linker to the thiadiazole. The *trans*-2-phenyl cyclopropyl-containing template **15** is 160 nM against dNEP with an *n*-propyl P_1'. The same feature in a methoxyethyl-containing molecule **16** is slightly more potent at 100 nM. A methoxymethyl group **17** improved potency slightly, but raised the possibility of β-elimination of methanol through removal of the proton adjacent to the acid. Nonetheless, it was clear that larger bulk adjacent to the amino-cyclic group, provided an appropriate linking scaffold was applied, or oxygenation of the α-carboxyl side chain could offer potency gains. A similar trend was seen in both the aminopyridine series of **18**, where a methoxyethyl side chain gave a 30-fold improvement in potency over propyl, and in the indanol series **19**, where the same change provided a 3-fold potency gain. We viewed the cyclopropane structures in particular as being quite different to the amino heterocycles such as **2** and as offering quite a bit of scope for further elaboration and potency optimisation. These were, therefore, followed up as top priority.

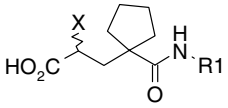
3.2. Cyclopropanes

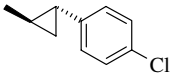
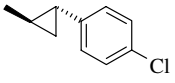
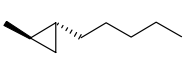
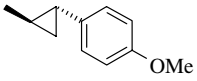
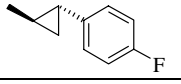
The *trans*-2-phenyl cyclopropane moiety of **15**–**17** showed reasonable potency in the 100 nM region,

similar to the starting point **2**. The methoxyethyl P_1' substituent was seen as one of the most versatile side chains, offering good potency in a relatively low lipophilicity structure, and was used to explore a number of cyclopropane ring substituents, including halogens, methoxy and an extended pentyl chain (Table 2). The chlorophenyl group was also made in the methoxymethyl P_1' series for comparison. In fact, the activity of the two P_1' series in this case was identical. Threefold to fourfold potency gains were made by substituting a halogen atom on the 4-position of the benzene ring. Most successful was substituting with a methoxy group which gave an approximately 10-fold gain in potency, as did the extended *n*-pentyl substituent. The clear message in this series was that potency gains could be made by extending functionality outwards from a suitably orientated amino-cyclic group. The origin of these potency gains is a better fit of these functionalities into a channel within the S_2' binding region, as evidenced by co-crystal structures of a variety of project compounds in a human NEP construct. A full account of this aspect of the project will be disclosed, elsewhere. Preparation of the amino-cyclopropanes required a rather lengthy synthesis, and ready access to chiral, non-racemic versions of both *cis*- and *trans*-amino-cyclopropanes was not trivial. We were also concerned about the chemical stability of these 1-amino-2-aryl-cyclopropanes and their potential to form reactive intermediates.

Small variations to the aminocyclopropanes which would produce much more synthetically accessible and chemically more robust targets were therefore investigated. The simplest and most attractive option was to break open the ring. This would produce aliphatic amide groups, which were structurally very different to the majority of the potent compounds we had encountered up to that point, but would still be able to access conformations and, therefore, activities of the cyclopropane analogues from which they were derived.

Table 2. Dog NEP activity of cyclopropyl glutaramides



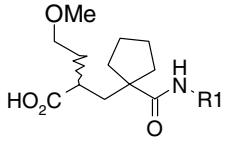
Compound	X	R^1	dNEP IC_{50} (nM)
20	Methoxymethyl		20
21	Methoxyethyl		21
22	Methoxyethyl		10
23	Methoxyethyl		8
24	Methoxyethyl		40

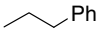
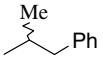
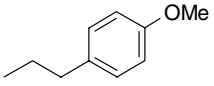
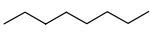
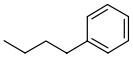
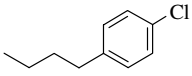
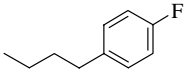
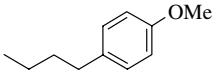
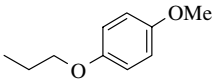
[a]Mean data of at least two determinations.
[b]All entries are racemic mixtures at both the P_1' centre and at the cyclopropane ring junctions. All compounds are *trans* diastereoisomers.
[c]All compounds showed >3 μM activity vs. pACE.

3.3. Aliphatic amides

Deleting the central cyclopropane ring carbon atom in **16** produced a 5-fold drop in binding potency, **25** (Table 3). Reducing the cyclopropane ring to produce an α-methyl phenethylamine **26** gave a similar drop in potency. Even reinstalling the most potent phenyl ring substituent, namely methoxy, into the phenethylamine template **27** did not alter potency greatly. Breaking open the cyclopropane ring of the pentyl-cyclopropane **28** largely retained potency, and hinted at there being much more volume within P_1' binding site to be accessed, which a phenethyl group was simply not reaching into. This assertion was borne out by extending the phenethylamine to a phenpropylamine **29** which improved potency by approximately 15-fold. Repeating the phenyl substitutions which had been successful in the case of the cyclopropanes above again gave excellent improve-

Table 3. Dog NEP activity of aliphatic glutaramides



Compound	R^1	dNEP IC_{50} (nM)
25		500
26		426
27		369
28		8
29		35
30		2
31		15
32		2
33		35

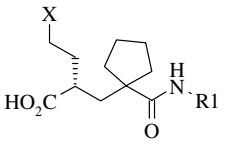
[a]Mean data of at least two determinations.
[b]All entries are racemic mixtures at the P_1' centre.
[c]All compounds showed >3 μM activity versus pACE.

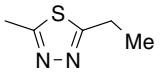
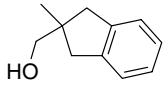
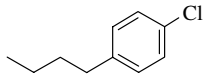
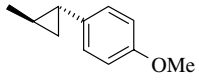
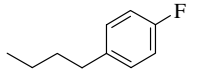
ments in potency. *p*-Chloro **30** and *p*-methoxy **32** were again particularly potent, although we did not favour the methoxy compound for fear of metabolic instability. The *p*-fluoro analogue **31** was some 7-fold less active than these compounds. Inserting an oxygen atom into the benzylic position of the propyl linker in **33** gave a drop of some 17-fold potency. These results indicated that the phenpropyl group occupied the binding site of NEP very much more effectively than shorter or smaller amide substituents, or heteroatom-linked substituents and that by appropriate substitution of the phenyl ring, large gains in binding potency could be realised. Very quickly we had arrived at low-nanometer potency, within the range of our criteria for a back-up agent to **2**.

3.4. Chiral, non-racemic compounds

Previous inhibitors from this series showed stereoselective binding, in favour of the *R*-enantiomer[3] (*R* for alkyl P_1' groups, and *S* for alkoxyalkyl groups, for example, methoxyethyl, both of which have the same stereochemical sense). A range of compounds from the above tables were therefore synthesised as single enantiomers and their activity against canine NEP confirmed (Table 4).

Table 4. Single enantiomer potency for selected compounds



Compound	X	R^1	dNEP IC_{50} (nM)
***R*-2**	Me		23.7
***S*-19**	OMe		5.4
***S*-30**	OMe		0.9
***S*-23**	OMe		8
***S*-31**	OMe		2

[a]Mean data of at least two determinations.
[b]All compounds showed >3 μM activity versus pACE and hECE-1.

Coupling of the corresponding phenpropyl amines to resolved versions of the acids **3** provided the single enantiomers of **2**, **19**, **30**, **23** and **31**. These compounds were indeed found to be significantly more active than the racemic mixtures, and again the phenpropyl compounds remained the most potent examples. Interestingly, in the case of the fluoro analogue ***S*-31**, the ratio of racemate potency to single enantiomer potency (7.5) was significantly greater than for the other two compounds. All compounds were shown to be completely selective for NEP over the related metallopeptidases ACE and ECE-1.

3.5. In vitro ADME data

All compounds from the above table were also progressed into a battery of in vitro tests to assess in vitro stability, permeability and physical properties, and compared with those of ***R*-2** (Table 5 below).

These data are interesting for a number of reasons. They offered a good range of compound properties with which to check our initial assertions in the project that a log *D* between 0 and 1, and Mwt below 400 would provide the best chance of achieving a good absorption profile. There is a relatively linear relationship between log *D* and Caco-2 permeability; setting a Caco-2 permeability of a compound which is predicted to be well absorbed in vivo at approximately 5, the data in the table suggest a minimum log *D* of ca. 0. This obviously distinguishes the polar ***S*-19** as poorly permeable and would therefore not be expected to be well absorbed in vivo. All compounds are very stable in HLM, although our assay conditions did not allow us to quantify the glucuronidation we expected in this series of carboxylic acids.[12] Table 5 clearly identifies the two halophenyl compounds ***S*-30** and *S*-**31** as the highest fluxed, and the most promising agents for progression. ***S*-23** is also well-fluxed, and interestingly our initial fears of the potential lability of the OMe group were not borne out by

Table 5. In vitro data for selected compounds

Compound	Mwt	log *D*[a]	Human PPB (%)[b]	HLM (min)[c]	Caco-2 P_{app} ($\times 10^{-6}$ cms^{-1})[d]	
					A–B	**B–A**
***R*-2**	339	0.5	97.9	>120	9	14
***S*-19**	389	−0.2	77.2	>120	1	2.5
***S*-30**	396	1.0	98.3	>120	12	14
***S*-23**	392	0.3	96.8	>120	7	ND
***S*-31**	379	0.5	87.6	>120	12	ND

[a] All log *D* measurements were made at pH 7.4 in *n*-octanol and phosphate buffer.
[b] Human plasma protein binding was determined in vitro at 1 μg/ml by equilibrium dialysis.
[c] Metabolic stability was determined in human liver microsomes at 1 μM compound concentration and 0.5 μM CYP concentration. The data are expressed as the first-order compound disappearance half-life.
[d] Caco-2 apparent permeability (P_{app}) was determined at pH 7.4 in both apical and basolateral chambers.

the HLM data for this compound. ***S*-19** was considered the least attractive compound because of its poor flux, but as will be seen below, low permeability in the Caco-2 model at pH 7.4 did not rule out complete in vivo absorption in this series of compounds.

3.6. pH differences and human NEP translation

The primary amino acid sequence of human NEP is >90% identical to that of other mammalian species, where the sequence is known. Sequence conservation is highest around the region which makes up the active site and at the outset of this work, we did not predict any particular differences in activity of our series of glutaramides in dog vs. human-derived NEP. Indeed, the first several compounds which were tested against both species of NEP showed very similar IC_{50} values such as ***R*-2** and ***S*-19**. It is notable that all compounds which feature an aliphatic or small cyclic amide substituent show similar or weaker potency against hNEP than against dNEP (***S*-30**, **23** and **31**), while compounds which show greater potency against hNEP feature a larger cyclic group attached to the amide N atom.

Clearly, those compounds which feature a larger cyclic group appended to the amide N atom will occupy different space and make a different set of interactions to the aliphatic compounds in the immediate vicinity of the amide linkage, which is possibly manifest as variant activity against the two NEP species.

***R*-2** and ***S*-30** were further assayed against rat, rabbit and recombinant hNEP to determine the extent of any additional variation in binding potency across these species (Table 7).

In the case of ***R*-2**, there is an approximately 2.6-fold maximum variation between the different species assay results which could be considered as being within the assay variability. For the second, aliphatic amide containing compound ***S*-30**, the variation is much bigger at approximately 8.6-fold at its maximum. Again, it seems that aliphatic amide groups are most prone to species variability.

During the course of these investigations into species differences, we had cause to look very carefully at the precise assay conditions which were being used to assess the potency of our compounds. The initial assay conditions used a pH of 7.0. Assay conditions were modified to produce a solution of pH 7.4 and the compounds of Table 6 were tested again for NEP activity in both human and dog assays (Table 8).

We found immediately that all compounds, without exception were less active in the pH 7.4 assay system than at pH 7.0. Typically, compounds were approximately 2–5 times less active at pH 7.4 than at pH 7.0. Upon closer inspection however, we noted that the drop in potency was mirrored across species, and had little effect on the rank order of potency, that is, the most potent compounds at pH 7.0 were still among the most potent at pH 7.4. In other words, the primary screening protocol which we used to prioritise compounds to be examined in our functional model of sexual arousal was unaffected by the pH variations. Figure 2 shows a plot of the canine and human NEP IC_{50}'s at the 2 pH's for a series of monoacid NEP inhibitors (not all data points are represented by the structures in Table 8; some are not shown in this paper).

There is an overall parallel shift of the data points away from the central line of complete correlation, indicating a consistent shift in IC_{50} values. In the case of hNEP, the shift was of the order of some 4.6-fold, whereas for dNEP it was about 2.8-fold. This effect has been noted before for NEP,[13] and for other metalloproteases such as ECE.[14]

Table 6. Comparison of hNEP and dNEP IC_{50}'s for selected compounds

Compound	hNEP IC_{50} (nM)	dNEP IC_{50} (nM)
***R*-2**	18.9	23.7
***S*-19**	3.7	5.4
***S*-30**	2.3	0.9
***S*-23**	6.1	1.5
***S*-31**	4.6	4.3

[a]Mean data of at least two determinations.

Table 7. IC_{50} of selected compounds against several species of NEP

Compound	HNEP IC_{50} (nM)	DNEP IC_{50} (nM)	ratNEP IC_{50} (nM)	rabbitNEP IC_{50} (nM)	rec-hNEP IC_{50} (nM)
***R*-2**	18.9	23.7	14.3	10.2	19.7
***S*-30**	2.3	0.9	0.6	0.8	5.4

[a]Mean data of at least two determinations.

Table 8. Comparison of hNEP and dNEP IC_{50}'s at pH 7.0 and 7.4

Compound	pH 7.0		pH 7.4	
	hNEP IC_{50} (nM)	dNEP IC_{50} (nM)	hNEP IC_{50} (nM)	dNEP IC_{50} (nM)
***R*-2**	18.9	23.7	31.8	ND
***S*-19**	3.7	5.4	10	14.2
***S*-30**	2.3	0.9	10.5	3.8
***S*-23**	6.1	1.5	13.8	5.7
***S*-31**	4.6	4.3	12.6	8.8

[a]Mean data of at least two determinations. ND, not determined.

Explanations of these pH differences are usually cited as being the difference in the extent of ionisation of the inhibitor or the enzyme. In the case of this series of inhibitors, the acid functional group has pK_a values of approximately 4.6 with little differences across the series, offering significantly more than 99% of ionised compound at both pH 7.0 and 7.4. The minute difference in ionised compound at pH 7.0 and 7.4 would not be expected to account for the differences seen above, unless uncharged compound contributed to NEP inhibition. The increase by 0.4 pH units reduces the amount of unionised compound by more than 2-fold. On the other hand, the active site of NEP does contain several highly basic residues, and also two histidine residues and a glutamic acid in the crucial vicinity of the zinc atom, and it seems to be more likely that small changes in the enzyme ionisation would better account for the observed potency differences, although evidence for this is lacking.[15] Since IC_{50} values for competitive inhibitors are dependent on the substrate concentration used in the assay relative to substrate affinity (Km), the large increase in IC_{50} with increasing pH could potentially be explained if the affinity for the substrate significantly increased with increasing pH. Our observations however (data not shown) do not support this explanation, since at pH 7.0, 7.4 and 8.0 we found the Km to be >100 μM, which is at least 5 times the substrate concentration we chose for the IC_{50} experiments. Interestingly, our Km estimate for the NEP substrate is significantly higher than the original published value of 2.8 μM.[9] Explanations for this might include a batch variation between the published peptide and the version we synthesised, or potential inaccuracies in determining the Km for fluorescent substrate of this type, which due to fluorescence inner filter effects can be distorted to appear lower than the true value. The effect of pH on the IC_{50} of ***S*-30** across a much bigger pH range was also examined. Across the range of Tris buffer (approximately pH 6.5–8.5), the IC_{50} was seen to increase with increasing pH in an apparently exponential fashion (Fig. 3).

The key issue, of course, was exactly what the most physiologically relevant pH was at which to measure compound potency. Our take on these observations was that the most relevant pH for this indication will most probably be pH 7.4. The pH of normal, healthy vasculature is 7.4, and even under conditions of stress

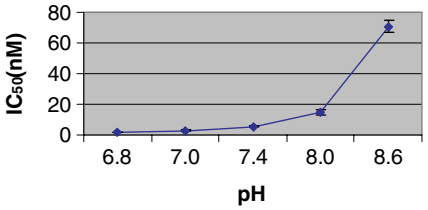


Figure 3. Potency variation of ***S*-30** with increasing pH (values are as follows: pH 6.8, IC_{50} 1.8 nM; 7.0, 2.9; 7.4, 5.2; 8.0, 15.0; 8.6, 70.9).

and/or exercise would not be expected to fall below this value by very much.

The assays described above consistently provide a rank order of compound potency at any given pH, to allow the selection of compounds for progression into animal models of sexual arousal and pharmacokinetic evaluation. Therefore, while worth noting, this phenomenon made no practical differences to the project.

3.7. Metabolic profile

Several compounds were evaluated in the rat, and their pharmacokinetic profiles measured (Table 9). All compounds displayed short $T_{1/2}$ and were also low Cl with high bioavailability. All compounds showed rapid absorption, with a T_{max} in each case of <1 h, that is, an ideal pharmacokinetic profile for *prn* dosing. This included ***S*-19**, which despite its polarity, and poor Caco-2 flux was well absorbed, which called into question the value of the Caco-2 model in predicting in vivo absorption in this series of compounds.[16]

It will be seen however, that across the compound range, there is some considerable variation in V_d, which is not expected from the physicochemical properties. We offer two reasonable hypotheses to explain this phenomenon.

First, reversible metabolism (Fig. 4) may contribute to the unusual pharmacokinetic profiles observed.[17]

Reversible metabolism occurs when a metabolite or biotransformation product and the parent drug undergo interconversion in both directions. All representatives of the glutarate series profiled underwent acyl glucuronidation. The acyl glucuronide can be excreted via the bile

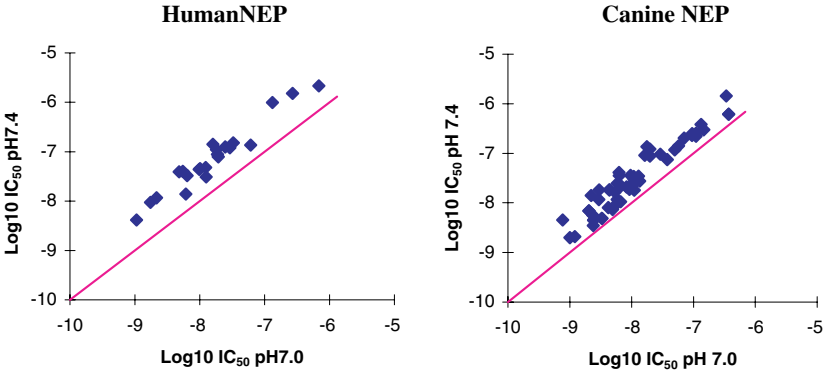


Figure 2. Comparison of pH 7.0 and 7.4 IC_{50}'s in human and dog NEP.

Table 9. Pharmacokinetics in rat of selected compounds

Compound	Mwt	log *D* pH 7.4	Cl (ml/min/kg)	V_d (L/kg)	$T_{1/2}$ (h)	f_u	*F* (oral) (%)
***R*-2**	339	0.5	3.0	0.44	1.7	0.106	103
***S*-19**	389	−0.2	18	7.5	4.8	0.088	109
***S*-30**	396	1.0	3.5	0.6	1.9	0.083	70
***S*-31**	379	0.5	13	2.7	2.4	0.138	138

[a]Jugular vein cannulated rats were dosed at 1 mg/kg iv (tail vein) and po (gavage). Blood samples were withdrawn via the indwelling catheter until 24 h post-dose.

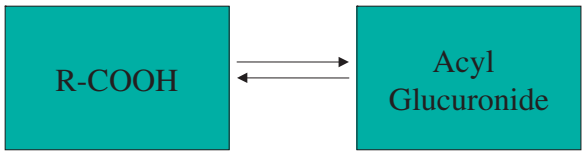


Figure 4. Reversible metabolism of carboxylic acids.

into the gut, where it will be chemically and/or enzymatically cleaved. The liberated parent is available for reabsorption (entero-hepatic cycling). It is likely that the secretion of the acyl glucuronide into the bile is mediated by MRP2, a carrier protein with high affinity to drug glucuronides.[18] Some acyl glucuronide will escape the biliary pathway and appear in the blood. There, it may be rapidly degraded and release parent depending on its chemical stability and its affinity to plasma esterases. For example, the decomposition half-life of the acyl glucuronide of ***R*-2** is ca. 5 min in dog, rat and human blood, whereas the acyl glucuronide of ***S*-30** does not degrade in blood from these species during 1 h at 37 °C. A fuller account of the profile and behaviour of the acyl glucuronides of this series of compounds will be reported elsewhere.

Hence, the pharmacokinetics of the glutaramides can be confounded by these two cyclic (reversible) processes. The irreversible clearance (Cl_{10}; cf. Fig. 5) of a glutarate can be achieved by non-UGT (i.e., CYP) mediated metabolism or by renal clearance of parent and/or acyl glucuronide (Cl_{20}).

From the above it is clear that the basic pharmacokinetic relationship Cl = Dose/AUC will provide only an apparent clearance value. This value will always underestimate the total elimination capacity for the drug, that is, $Cl_{10} + Cl_{12}$. The apparent clearance value observed will be the more inaccurate, the greater the extent of Cl_{12}, that is, the greater the contribution of acyl glucuronidation to total clearance. In species with high glucuronidation capacity (such as dog[19]) and in species with a low threshold for biliary excretion (such as rat and dog[20]) the apparent clearance will particularly underestimate the true elimination capacity. The pharmacokinetics of those carboxylic acids that are predominantly glucuronidated (such as ***S*-30** and ***S*-31**) will suffer significantly more from this complication than compounds with minute to moderate acyl glucuronidation. In humans, where the biliary threshold tends to be higher and the glucuronidation capacity is usually lower than in dog, reversible kinetics will have less of an influence. Similar considerations can be made for the second primary PK parameter, the volume of distribution (V_d).

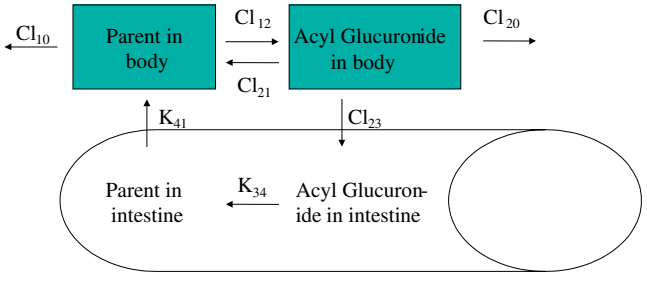


Figure 5. Clearance terms and rate constants in the PK of carboxylic acids.

Second, active concentrative hepatic uptake of compounds can lead to a volume of distribution larger than expected from their physicochemistry.[21] This is particularly evident for acidic compounds. For example, the acidic NSAID ketoprofen is actively taken up into rat liver[22] which results in a volume of distribution between 0.8 and 1.1 L/kg,[23] considerably larger than the 0.2 L/kg expected from physicochemistry. Similar observations have also been made for a dicarboxylic acid endothelin receptor antagonist.[24] Taking into account these acid-specific phenomena, ***S*-30** was chosen to further progress into a model of sexual arousal on the basis of low unbound clearance and potency. These results will be presented in due course.

4. Conclusion

We have shown that relatively small (Mwt < 400) substituted phenpropylamides with halide or alkoxy substituents are potent and selective inhibitors of NEP. Incorporation of various substitution patterns at the P_1' position is tolerated, and changes in this region, along with changes in the substituents on the phenpropylamine fragment, allowed a range of compounds to be prepared with disparate log *D*. Profiling of some of these compounds in animal models has suggested these NEP inhibitors should be efficacious with a pharmacokinetic profile which is suited to *prn* dosing in humans. Of these compounds, ***S*-30** was chosen for further study.

5. Experimental

5.1. Chemistry

Melting points were determined on a Gallenkamp Melting point apparatus using glass capillary tubes and are uncorrected. Unless otherwise indicated all reactions

were carried out under a nitrogen atmosphere, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed precoated Merck silica gel (60 F254) plates, and flash chromatography was carried out using 40–63 μm silica gel. Proton NMR spectra were measured on a Varian Inova 300 or 400 spectrometer in the solvents specified. Mass spectra were recorded on a Fisons Trio 1000 using thermospray positive (TSP) ionisation. Combustion analyses were conducted by Exeter Analytical UK. Ltd, Uxbridge, Middlesex. The purity of compounds was also carefully assessed using analytical TLC and proton NMR, the latter technique was used to calculate the amount of solvent in solvated samples. In multistep sequences, the purity and structure of intermediates were verified spectroscopically by ^{1}H NMR. Optical rotations were determined using a Perkin-Elmer 341 polarimeter. Optical purity was determined by analytical HPLC analysis of classically resolved material (see Section 5 below) via comparison to racemic material. Unless otherwise stated, all starting materials and solvents were readily available from commercial suppliers. The preparation of compounds **2**, **14**, **18** and **19** has been described previously.[3b] Compounds **15**–**17**, **22**, **25** and **27**–**29** were all prepared from commercially available amines. Compound **9** (R = Cl) was prepared by the literature method.[9]

5.2. NEP inhibition assay

In a 96-well microtitre plate 100 μl of fluorescent NEP substrate peptide (50 μM *o*-aminobenzoyl-*d*-Arg-Arg-Leu-ethylenediamine 2,4-dinitrophenyl) was added to 50 μl of 4 times test concentration (4×) inhibitor solutions. The assays were initiated by the addition of 50 μl of enzyme which had been previously diluted such that with an incubation time of 1 h the percentage of substrate converted to product was approximately 10% or less. The reaction mixture was incubated for 1 h at 37 °C in a shaking incubator, then stopped by the addition of 100 μl of 300 nM phosphoramidon. Samples corresponding to 100% substrate to product conversion were included to enable the percent substrate proteolysed to be determined. Each assay point was in duplicate. Each IC_{50} experiment used a 10 point dilution series at half-logarithmic inhibitor concentration increments, with the highest concentration either 10, 1 or 0.1 μM. After the 1 h incubation, fluorescence of the samples was measured (Ex320/Em420) using a BMG Fluostar Galaxy reader and an IC_{50} calculated using a customised Microsoft Excel add-inn. Enzyme and substrate were prepared in 50 mM Tris–Cl (pH 7.4 at 37 °C), and 4× inhibitor dilutions were made in 50 mM Tris–Cl, pH 7.4, at 37 °C/4% DMSO. In the experiments where the pH of the assay was modified, enzyme, substrate and inhibitor solutions were all prepared in Tris buffer at the appropriate pH. The range of pH validated pre-set buffer crystals (Sigma, UK) was used for this. Standard compounds assessed in this assay were as follows; phosphoramidon 0.71 nM, thiorphan 3.85 nM and candoxatrilat 2.61 nM. IC_{50} estimates stated for NEP represent the geometric mean of at least two values obtained using separately weighed and solubilised compound samples. The NEP assay was highly reproducible. We demonstrated this from a statistical analysis of 25 IC_{50} values obtained from a single compound tested multiple times over an 8-month period. By assessing the assay-to-assay variation we were able to conclude that two replicates would be sufficient to ensure that the geometric mean IC_{50} was estimated with less than 2-fold error, based on the width of the 95% confidence interval.

5.3. ACE inhibition assay

Performed as described above for the NEP assay, except the substrate used was *o*-aminobenzoyl-Gly-Gly-*p*-nitro-Phe-Pro-OH, at a final assay concentration of 10 μM, the enzyme and substrate buffer was 50 mM Tris–Cl, pH 7.4 (at 37 °C), 300 mM NaCl, and the reaction was terminated using 100 μl of a 2 mM EDTA solution.

5.4. ECE-1 inhibition assay

Performed as described above for the ACE assay.

5.5. Pharmacokinetic studies

Male CD rats (Charles River, Manston, UK) were used. These were jugular vein cannulated under general anaesthesia, with the cannula exteriorised at the back of the neck. At specified times after dosing, 0.2 ml samples of blood were withdrawn from the cannula and transferred to heparin tubes. Following adequate mixing the blood samples were centrifuged and plasma was generated. Immediately after that plasma was stabilised by addition of 0.01 parts of phosphoric acid (85%, v/v) and stored deep frozen until analysis. The fraction unbound in rat plasma was determined at 1 μg/ml using equilibrium dialysis against isotonic Krebs–Ringer bicarbonate buffer (pH 7.4). The concentrations of glutaramides in rat plasma were determined by LC–MS–MS (Hewlett Packard HP1100 binary HPLC pump, CTC Pal Autosampler, Sciex API 2000 mass spectrometer with TurboIonSpray interface) at a flow rate of 1 ml/min, split 50:1 post-column using an Acurate™ flow splitter. Glutaramides and internal standard (see Table 10) were extracted from 100 μl of acidified plasma mixed with 400 μl of 50 mM ammonium formate buffer (pH 3.5) using activated C18 IST cartridges in 96-well format (Porvair). The cartridges were washed with 1 ml of 50 mM ammonium formate (pH 3.5). Compounds were eluted with 1 ml of acetonitrile containing 5% (v/v) formic acid and evaporated to dryness under nitrogen at 20 °C. The residues were resuspended in 200 μl of 2 mM ammonium acetate in 70:30 (v/v) methanol/water (pH 3.5) and 180 μl was injected into the HPLC-mass spectrometer. The mobile phase was 2 mM ammonium acetate in methanol/water (90:10; v/v; pH 3.5) and the column was a Hypersil HS100 C18, 5 μm, 50 × 4.6 mm. Detection was carried out by positive ion multiple reaction monitoring (MRM) at Q1 and Q3 resolution of ca. 0.7 Da peak width at half-height (Table 10). The curtain gas, nebuliser gas and TurboIonSpray gas was nitrogen at settings of 30 (CUR), 25 (GS1) and 40 (GS2), respectively. TurboIonSpray temperature was 100 °C. The collision gas was nitrogen at a setting of

Table 10. MRM transitions for the plasma analysis of glutaramides

Compound	Q1	Q3
***R*-2**	340	130
***S*-9**	390	164
***S*-20**	396	346
***S*-21**	380	330
22	392	342
4	418	192
15	326	116

2. The collision energy was 25 eV (OR 30 V), the dwell time was 200 ms with 50 ms pause.

All other data were obtained using standard methods.

5.6. General procedure 1. Amide formation

The requisite amine derivative (1 equiv) and the acid **3a**–**f**[3,7] (1 equiv) were dissolved in DCM or MeCN (1 M) and NMM (1 equiv) added in one portion. HOBt (1 equiv) was then added, followed by 1 equiv of WSCDI. The mixture was stirred at room temperature (except in the case of less reactive amino heterocycles viz. *tert*-butyl-**2**, -**14** and -**18** or hindered viz. *tert*-butyl-**19** amines which were heated at 100 °C in DMF) for 12 h under a nitrogen atmosphere, or until reaction was complete as judged by TLC analysis. The mixture was diluted with EtOAc and washed several times with water. The organic layer was separated, washed again with water, dried ($MgSO_4$) and evaporated to afford the desired amides, which were purified by flash chromatography using mixtures of either pentane and EtOAc or MeOH and DCM (typically between 30% and 50% EtOAc in pentane or between 2% and 5% MeOH in DCM).

The following amides were prepared as protected forms of the final target structures according to

5.6.1. General procedure 1

5.6.1.1. 2-[1-(5-Ethyl-[1,3,4]thiadiazol-2-ylcarbamoyl)-cyclopentylmethyl]-pentanoic acid *tert*-butyl ester, *tert*-butyl-2. δ_H ($CDCl_3$, 300 MHz) 0.82 (t, J = 7.0 , 3H), 1.20–1.80 (m, 22H), 1.84–1.87 (m, 1H), 2.18–2.23 (m, 4H), 3.04 (q, J = 7.5 Hz, 2H), 9.10 (br s, 1H). LRMS: m/z (TS^+) 396 (M+H). Yield 92%.

5.6.1.2. 2-[1-(5-Benzyl-[1,3,4]thiadiazol-2-ylcarbamoyl)-cyclopentylmethyl]-4-methoxy-butyric acid *tert*-butyl ester, *tert*-butyl-14. δ_H ($CDCl_3$, 400 MHz) 1.32 (s, 9H), 1.54–2.31 (m, 13H), 3.20 (s, 3H), 3.25–3.33 (m, 2H), 4.30 (s, 2H), 7.22–7.33 (m, 5H), 10.49 (br s, 1H). LRMS: (ES^+) 474 (M+H). Yield 88%.

5.6.1.3. 2-[1-(*trans*-2-Phenyl-cyclopropylcarbamoyl)-cyclopentylmethyl]-pentanoic acid *tert*-butyl ester, *tert*-butyl-15. δ_H ($CDCl_3$, 400 MHz) 0.88 (t, J = 6.9 Hz, 3H), 1.16–1.20 (m, 1H), 1.20–1.58 (m, 16H), 1.63–1.66 (m, 5H), 1.90–2.14 (m, 4H), 2.23–2.25 (m, 1H), 2.90–2.93 (m, 1H), 6.00–6.06 (m, 1H), 7.16–7.22 (m, 3H), 7.24–7.27 (m, 2H). LRMS: m/z (ES^+) 400 (M+H). Yield 86%.

5.6.1.4. 4-Methoxy-2-[1-(*trans*-2-phenyl-cyclopropylcarbamoyl)-cyclopentylmethyl]-butyric acid *tert*-butyl ester, *tert*-butyl-16. δ_H ($CDCl_3$, 400 MHz) 1.10–1.14 (m, 1H), 1.16–1.20 (m, 1H), 1.42 (s, 9H), 1.58–1.67 (m, 5H), 1.70–1.72 (m, 1H), 1.89–1.91 (m, 2H), 2.02–2.10 (m, 2H), 2.43 (q, J = 6.8 Hz, 1H), 2.83 (q, J = 6.8 Hz, 1H), 3.29 (s, 3H), 3.11–3.18 (m, 2H), 6.15 (br s, 1H), 7.09–7.23 (m, 5H). LRMS: (TS^+) 416 (M+H). Anal. Found C, 71.61; H, 9.01; N, 3.36%. $C_{25}H_{37}NO_4$ requires C, 71.94; H, 8.98; N, 3.36%. Yield 60%.

5.6.1.5. 2-Methoxymethyl-3-[1-(*trans*-2-phenyl-cyclopropylcarbamoyl)-cyclopentyl]-propionic acid *tert*-butyl ester, *tert*-butyl-17. δ_H ($CDCl_3$, 400 MHz) 1.00–1.10 (m, 2H), 1.40 (s, 9H), 1.50–1.80 (m, 6H), 1.80–2.05 (m, 4H), 2.30–2.40 (m, 1H), 2.70–2.80 (m, 1H), 3.20 (s, 3H), 3.25–3.35 (m, 1H), 3.70 (s, 3H), 6.05 (s, 1H), 6.70–7.05 (m, 5H). LRMS: m/z (TS^+) 446 (M+H). Yield 62%.

5.6.1.6. 2-Methoxymethyl-3-[1-(*trans*-2-phenyl-cyclopropylcarbamoyl)-cyclopentyl]-propionic acid benzyl ester, 10. δ_H ($CDCl_3$, 400 MHz) 1.00–1.06 (m, 1H), 1.22 (dd, J = 10.4, 6.8 Hz, 1H), 1.37–1.48 (m, 2H), 1.60–2.10 (m, 10H), 2.52–2.59 (m, 1H), 2.77–2.84 (m, 1H), 3.22 (s, 3H), 3.37 (t, J = 6.5 Hz, 2H), 5.03 (s, 2H), 5.90 (s, 1H), 7.11–7.32 (m, 10H). Anal. Found C, 74.17; H, 7.90; N, 3.17%. $C_{28}H_{35}NO_4$ requires C, 74.21; H, 7.87; N, 3.09%. Yield 74%.

5.6.1.7. 2-[1-(4-Butyl-pyridin-2-ylcarbamoyl)-cyclopentylmethyl]-4-methoxybutyric acid benzyl ester, Bn-18. δ_H ($CDCl_3$, 400 MHz) 0.89 (t, J = 6.9 Hz, 3H), 1.24–1.32 (m, 2H), 1.47–2.26 (m, 14H), 2.55–2.68 (m, 3H), 3.17 (s, 3H), 3.24 (t, J = 7.3 Hz, 2H), 4.99 (s, 2H), 6.83 (d, J = 5.3 Hz, 1H), 7.27–7.35 (m, 5H), 7.94 (br s, 1H), 8.07 (s, 1H), 8.13 (d, J = 5.4 Hz, 1H). Yield 66%.

5.6.1.8. 2-[1-(2-Hydroxymethyl-indan-2-ylcarbamoyl)-cyclopentylmethyl]-4-methoxy-butyric acid *tert*-butyl ester, *tert*-butyl-19. δ_H ($CDCl_3$, 400 MHz) 1.40 (s, 9H), 1.44–2.00 (m, 12H), 2.37–2.43 (m, 1H), 2.99 (d, J = 15.6 Hz, 1H), 3.08 (d, J = 15.6 Hz, 1H), 3.20–3.38 (m, 7H), 3.65 (dd, J = 6.8, 0.8 Hz, 2H), 4.40 (br s, 1H), 6.00 (s, 1H), 7.10–7.18 (m, 4H). Yield 66%.

5.6.1.9. 3-{1-[2-(*trans*-4-Chlorophenyl)-cyclopropylcarbamoyl]-cyclopentyl}-2-methoxymethyl-propionic acid *tert*-butyl ester, *tert*-butyl-20. δ_H ($CDCl_3$, 400 MHz) 1.15–1.30 (m, 4H), 1.50–1.70 (m, 1H), 1.80–1.90 (m, 1H), 2.40–3.55 (m, 1H), 4.20 (dd, J = 5.7, 1.1 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 7.20–7.28 (m, 2H). HRMS: m/z Found 436.2242. $C_{24}H_{35}NO_4Cl$ requires M+H. 436.2249, Yield 40%.

5.6.1.10. 2-{1-[2-(*trans*-4-Chlorophenyl)-cyclopropylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid *tert*-butyl ester, *tert*-butyl-21. δ_H ($CDCl_3$, 400 MHz) 1.10–1.19 (m, 2H), 1.42 (s, 9H), 1.57–1.82 (m, 8H), 1.85–2.05 (m, 5H), 2.33–2.43 (m, 1H), 2.77–2.81 (m, 1H), 3.23 (s, 3H), 3.39 (dd, J = 5.7, 1.0 Hz, 2H), 6.05 (br s, 1H), 7.14 (d, J = 8.8 Hz, 2H), 7.28 (d, J = 8.8 Hz, 2H). LRMS: m/z (TS^+) 450 (M+H). Yield 86%.

5.6.1.11. 4-Methoxy-2-[1-(*trans*-2-pentyl-cyclopropylcarbamoyl)-cyclopentylmethyl]-butyric acid *tert*-butyl ester, *tert*-butyl-22. δ_H ($CDCl_3$, 400 MHz) 0.40–0.60 (m, 2 H), 0.70–0.90 (m, 4H), 1.05–1.15 (m, 1H), 1.20–1.30 (m, 4H), 1.30–1.50 (m, 14H), 1.50–2.00 (m, 10H), 2.20–2.40 (m, 2H), 3.20–3.35 (m, 5H), 5.70–5.90 (br s, 1H). LRMS: *m*/*z* (TS^+) 410 (M+H). Yield 71%.

5.6.1.12. 4-Methoxy-2-{1-[(*trans*-2-(4-methoxy-phenyl)-cyclopropylcarbamoyl)]-cyclopentylmethyl}-butyric acid *tert*-butyl ester, *tert*-butyl-23. δ_H ($CDCl_3$, 400 MHz) 1.00–1.10 (m, 2H), 1.40 (s, 9H), 1.35–1.45 (m, 2H), 1.50–1.80 (m, 7H), 1.85–2.10 (m, 4H), 2.25–2.35 (m, 1H), 2.70–2.80 (m, 1H), 3.20 (s, 3H), 3.25–3.35 (m, 2H), 3.70 (s, 3H), 6.05 (br s, 1H), 6.70 (d, J = 8.6 Hz, 2H), 7.05 (d, J 8.6 Hz, 2H). LRMS: *m*/*z* (TS^+) 446 (M+H). Yield 38%.

5.6.1.13. 2-{1-[(*trans*-2-(4-Fluoro-phenyl)-cyclopropylcarbamoyl)]-cyclopentylmethyl}-4-methoxy-butyric acid *tert*-butyl ester, *tert*-butyl-24. δ_H ($CDCl_3$, 400 MHz) 1.10–1.20 (m, 2H), 1.42 (s, 9H), 1.55–1.82 (m, 8H), 1.97–2.12 (m, 5H), 2.33–2.43 (m, 1H), 2.77–2.88 (m, 1H), 3.24 (s, 3H), 3.37–3.45 (m, 2H), 6.05 (br s, 1H), 6.96 (d, J = 8.6 Hz, 2H), 7.16–7.20 (m, 2H). LRMS: *m*/*z* (TS^+) 434 (M+H). Yield 65%.

5.6.1.14. 4-Methoxy-2-(1-phenethylcarbamoyl-cyclopentylmethyl)-butyric acid benzyl ester, Bn-25. δ_H ($CDCl_3$, 400 MHz) 1.28–2.03 (m, 10H), 2.43–2.57 (m, 1H), 2.78 (t, J = 6.9 Hz, 2H), 3.20 (s, 3H), 3.24 (t, J = 6.8 Hz, 2H), 3.39–3.50 (m, 2H), 5.03 (s, 2H), 5.64 (br s, 1H), 7.16–7.37 (m, 10H). Yield 65%.

5.6.1.15. 4-Methoxy-2-{1-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-cyclopentylmethyl}-butyric acid *tert*-butyl ester, *tert*-butyl-27. ^{1}H NMR (400 MHz, $CDCl_3$) 1.42 (s, 9H), 1.60–1.64 (m, 5H), 1.83–1.89 (m, 4H), 2.00–2.04 (m, 1H), 2.33–2.37 (m, 1H), 2.68 (t, J = 6.9 Hz, 2H), 3.27 (s, 3H), 3.37–3.39 (m, 2H), 3.77 (s, 3H), 5.73 (br s, 2H), 6.85 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.6 Hz, 2H). LRMS: (ES^-) 448 (M−H). Yield 65%.

5.6.1.16. 2-(1-Heptylcarbamoyl-cyclopentylmethyl)-4-methoxy-butyric acid *tert*-butyl ester, *tert*-butyl-28. δ_H ($CDCl_3$, 300 MHz) 0.82 (t, J = 6.7 Hz, 3H), 1.15–1.30 (m, 8H), 1.42 (s, 9H), 1.40–1.50 (m, 4H), 1.50–2.00 (m, 11H), 2.30–2.40 (m, 1H), 3.09–3.23 (m, 2H), 3.20 (s, 3H), 3.30 (t, J = 6.5 Hz, 2H), 5.70 (br s, 1H). Yield 86%.

5.6.1.17. 4-Methoxy-2-[1-(3-phenyl-propylcarbamoyl)-cyclopentylmethyl]-butyric acid *tert*-butyl ester, *tert*-butyl-29. δ_H ($CDCl_3$, 300 MHz) 1.40 (s, 9H), 1.45 (m, 2H), 1.60 (m, 8H), 1.80 (m, 4H), 2.30 (m, 1H), 2.60 (t, J = 6.7 Hz, 2H), 3.20 (s, 3H), 3.30 (m, 4H), 3.80 (br s, 1H), 7.10 (d, J = 8.6 Hz, 3H), 7.20 (d, J = 8.6 Hz, 2H). LRMS: *m*/*z* (ES^-) 418 (M−H). Yield 76%.

5.6.1.18. 2-{1-[3-(4-Chloro-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid *tert*-butyl ester, *tert*-butyl-30. δ_H ($CDCl_3$, 400 MHz) 1.40 (s, 10H), 1.50–2.05 (m, 13H), 2.30–2.40 (m, 1H), 2.60 (t, J = 6.7 Hz, 2H), 3.25 (s, 3H), 3.20–3.35 (m, 4H), 5.80 (br s, 1H), 7.10 (d, J = 8.6 Hz, 2H), 7.20 (d, J = 8.6 Hz, 2H). LRMS: *m*/*z* (TS^+) 452 (M+H). Yield 68%.

5.6.1.19. 2-{1-[3-(4-Fluoro-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid *tert*-butyl ester, *tert*-butyl-31. ^{1}H NMR (400 MHz, $CDCl_3$) 1.42 (s, 9H), 1.50–1.53 (m, 2H), 1.60–1.62 (m, 4H), 1.74–1.82 (m, 5H), 1.89–1.93 (m, 3H), 2.27–2.31 (m, 1H), 2.63 (t, J = 6.7 Hz, 2H), 3.08–3.13 (m, 1H), 3.21 (s, 3H), 3.38 (t, J = 6.8 Hz, 2H), 5.88–5.90 (m, 1H), 6.91 (d, J = 8.6 Hz, 2H), 7.15 (d, J = 8.6 Hz, 2H). LRMS: (ES^+) 436 (M+H). Yield 67%.

5.6.1.20. 4-Methoxy-2-{1-[3-(4-methoxy-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-butyric acid *tert*-butyl ester, *tert*-butyl-32. ^{1}H NMR (400 MHz, $CDCl_3$) 1.42 (s, 9H), 1.60–1.65 (m, 5H), 1.84–1.86 (m, 4H), 2.08–2.15 (m, 1H), 2.28–1.33 (m, 1H), 2.66 (t, J = 6.8 Hz, 2H), 3.26 (s, 3H), 3.26–3.34 (m, 2H), 3.74 (s, 3H), 5.74 (br s, 1H), 6.84 (d, J = 8.6 Hz, 2H), 7.16 (d, J = 8.6 Hz, 2H). LRMS: (ES^-) 448 (M−H). Yield 85%.

5.6.1.21. 4-Methoxy-2-{1-[2-(4-methoxy-phenoxy)-ethylcarbamoyl]-cyclopentylmethyl}-butyric acid *tert*-butyl ester, *tert*-butyl-33. ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.42 (s, 9H), 1.41–1.44 (m, 2H), 1.63–1.69 (m, 6H), 1.72–1.75 (m, 2H), 1.98–2.05 (m, 3H), 2.36–2.39 (m, 1H), 3.17 (s, 3H), 3.25 (t, J = 6.8 Hz, 2H), 3.57–3.62 (m, 2H), 3.78 (s, 3H), 3.91 (t, J = 6.8 Hz, 2H), 6.10–6.12 (m, 1H), 6.71 (m, 4H). LRMS: (TS^+) 450 (M+H). HRMS: Found 450.2842. $C_{25}H_{39}NO_6$ requires M+H, 450.2840. Yield 60%.

5.7. General procedure 2. *tert*-Butyl ester deprotection

The requisite *tert*-butyl ester (1 equiv) was dissolved in a 1:1 (v/v) mixture of TFA and DCM (0.25 M total) and the reaction mixture stirred at room temperature under a nitrogen atmosphere overnight. The reaction mixture was evaporated and then partitioned between DCM and water. The organic layer was separated, washed several times with water, dried ($MgSO_4$), then evaporated and the residue purified by flash chromatography on silica gel using mixtures of between 2% and 5% MeOH and DCM to afford the desired acid. Alternatively, the *tert*-butyl ester was dissolved in either Et_2O, DCM or EtOAc, and HCl gas bubbled through the solution at room temperature for 10 min. The solution was then purged by bubbling through N_2 gas for a further 10 min and then evaporated to dryness. The residue was purified by flash chromatography on silica gel as above to afford the desired acids.

The following acids were prepared according to

5.7.1. General procedure 2

5.7.1.1. 2-[1-(5-Ethyl-[1,3,4]thiadiazol-2-ylcarbamoyl)-cyclopentylmethyl]-pentanoic acid, 2. δ_H ($CDCl_3$, 400 MHz) 0.92 (t, 3H, J = 7.0 Hz), 1.35 (t, 3H, J = 7.5 Hz), 1.25–1.80 (m, 11H), 2.20–2.50 (m, 4H), 2.95 (q, 2H, J = 7.5 Hz), 12.10 (br s, 1H); LRMS *m*/*z* (TS^+) 340 (M+H); Anal. Found C, 56.46; H, 7.46; N, 12.37%. ($C_{16}H_{25}N_3O_3S$) requires C, 56.62; H, 7.44; N, 12.37%. Yield 86%.

5.7.1.2. 2-[1-(5-Benzyl-[1,3,4]thiadiazol-2-ylcarbamoyl)-cyclopentylmethyl]-4-methoxy-butyric acid, 14. δ_H ($CDCl_3$, 400 MHz) 0.62–2.57 (m, 13H), 3.07 (s, 3H), 2.96–3.44 (m, 2H), 4.09 (s, 2H), 7.20 (br s, 5H). HRMS: *m/z* Found 418.1796. $C_{21}H_{27}N_3O_4S$ requires M^+ 418.1795. Anal. Found C, 60.63; H, 6.44; N, 10.37%. ($C_{21}H_{27}N_3O_4S$) requires C, 60.41; H, 6.52; N, 10.06%. Yield 64%.

5.7.1.3. 2-[1-(*trans*-2-phenyl-cyclopropylcarbamoyl)-cyclopentylmethyl]-pentanoic acid, 15. δ_H ($CDCl_3$, 400 MHz) 0.90 (t, 3H, J = 6.9 Hz), 1.12–2.14 (m, 17H), 2.32–2.42 (m, 1H), 2.84–2.92 (m, 1H), 6.10 (s, 1H), 7.09–7.17 (m, 3H), 7.20–7.30 (m, 2H). LRMS: *m/z* (TS^+) 344 (M+H). Yield 86%.

5.7.1.4. 4-Methoxy-2-[1-(*trans*-2-phenyl-cyclopropylcarbamoyl)-cyclopentylmethyl]-butyric acid, 16. δ_H ($CDCl_3$, 400 MHz) 1.02–1.26 (m, 2H), 1.37–1.84 (m, 7H), 1.85–2.16 (m, 4H), 2.62 (br s, 1H), 2.80–2.93 (m, 1H), 3.29 (s, 3H, Me), 3.22–3.58 (m, 2H), 6.21 (br s, 1H), 7.03–7.34 (m, 5H). LRMS: *m/z* (TS^+) 346 (M+H). HRMS: *m/z* Found 346.2011. $C_{20}H_{27}NO_4$ requires M+H. 346.2013. Anal. Found C, 69.51; H, 7.86; N, 4.37%. ($C_{20}H_{27}NO_4$) requires C, 69.54; H, 7.88; N, 4.05%. Yield 88%.

5.7.1.5. 2-Methoxymethyl-3-[1-(*trans*-2-phenyl-cyclopropylcarbamoyl)-cyclopentyl]-propionic acid, 17. δ_H ($CDCl_3$, 400 MHz) 1.13–1.28 (m, 2H), 1.48–1.56 (m, 3H), 1.60 (br s, 3H), 1.81 (d, 1H), 2.01–2.06 (m, 4H), 2.61 (br s, 1H), 2.93 (br s, 1H), 3.30 (s, 3H), 3.42 (d, 1H, J = 9.3 Hz), 3.52 (d, 1H, J = 9.3 Hz), 6.40 (s, 1H), 7.08–7.16 (m, 3H), 7.26–7.37 (m, 2H). LRMS: *m/z* (ES^-) 344 (M−H). Anal. Found C, 69.44; H, 7.80; N, 4.01%. $C_{20}H_{27}NO_4$ requires C, 69.54; H, 7.88; N, 4.05%. Yield 45%.

5.7.1.6. 2-[1-(2-Hydroxymethyl-indan-2-ylcarbamoyl)-cyclopentylmethyl]-4-methoxy-butyric acid 19. δ_H ($CDCl_3$, 400 MHz) 1.43–1.76 (m, 7H), 1.80–2.24 (m, 4H), 2.57–2.68 (m, 2H), 3.06 (d, 1H J = 16.5 Hz), 3.12 (d, 1H J = 15.7 Hz), 3.27–3.31 (m, 1H), 3.32 (s, 3H), 3.36–3.48 (m, 2H), 3.80 (d, 1H J = 6.5 Hz), 3.87 (d, 1H J = 6.5 Hz), 6.04 (s, 1H), 7.16–7.22 (m, 4H). Anal. Found C, 70.61; H, 8.37; N, 3.74%. $C_{22}H_{31}NO_4$ requires C, 70.75; H, 8.37; N, 3.75%. Yield 89%.

5.7.1.7. 3-{1-[2-(*trans*-4-Chlorophenyl)-cyclopropylcarbamoyl]-cyclopentyl}-2-methoxymethyl-propionic acid, 20. δ_H ($CDCl_3$, 400 MHz) 1.04–1.18 (m, 2H), 1.20–1.36 (m, 2H), 1.36–1.79 (m, 7H), 1.83–2.08 (m, 4H), 2.57–2.66 (m, 1H), 2.73–2.83 (m, 1H), 3.27 (s, 3H), 3.38 (t, 1H J = 9.3 Hz), 3.49 (t, 1H J = 9.3 Hz), 6.21 (br s, 1H), 7.03 (d, 2H J = 8.8 Hz), 7.18 (d, 2H J = 8.8 Hz). LRMS: *m/z* (ES^-) 378 (M−H). HRMS: *m/z* Found 380.1622. $C_{20}H_{26}ClNO_4$ requires MH^+ 380.1623. Yield 12%.

5.7.1.8. 2-{1-[2-(*trans*-4-Chlorophenyl)-cyclopropylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid, 21. δ_H ($CDCl_3$, 400 MHz) 1.10–1.20 (m, 2H), 1.50–1.70 (m, 8H), 1.85–2.05 (m, 5H), 2.55–2.65 (m, 1H), 2.72–2.85 (m, 1H), 3.20 (s, 3H), 3.31–3.43 (m, 2H), 6.00 (s, 1H), 7.12 (d, J = 8.6 Hz, 2H), 7.23 (d, J = 8.6 Hz, 2H). LRMS: *m/z* (ES^-) 392 (M−H). Anal. Found C, 63.91; H, 7.10; N, 3.53%. $C_{21}H_{28}ClNO_4$ requires C, 64.03; H, 7.16; N, 3.56%. Yield 74%.

5.7.1.9. 4-Methoxy-2-[1-(*trans*-2-pentyl-cyclopropylcarbamoyl)-cyclopentylmethyl]-butyric acid, 22. δ_H ($CDCl_3$, 400 MHz) 0.50–0.63 (m, 3H), 0.77–0.84 (m, 4H), 1.01–1.18 (m, 1H), 1.20–1.78 (m, 14H), 1.82–2.08 (m, 2H), 3.27 (s, 3H), 3.33–3.41 (m, 2H), 5.92 (s, 1H). LRMS: *m/z* (ES^-) 352 (M−H). Anal. Found C, 67.66; H, 10.04; N, 4.13%. ($C_{20}H_{35}NO_4$) requires C, 67.95; H, 9.98; N, 3.96%. Yield 47%.

5.7.1.10. 4-Methoxy-2-{1-[(*trans*-2-(4-methoxy-phenyl)-cyclopropylcarbamoyl)]-cyclopentylmethyl}-butyric acid, 23. δ_H ($CDCl_3$, 400 MHz) 1.01–1.22 (m, 2H), 1.40–2.22 (m, 15H), 2.42–2.57 (m, 1H), 2.73–2.82 (m, 1H), 3.23 (s, 3H), 3.27–3.44 (m, 2H), 3.72 (s, 3H), 6.12 (s, 1H), 6.78 (d, J = 8.6 Hz, 2H), 7.06 (d, J = 8.6 Hz, 2H). LRMS: *m/z* (ES^-) 388 (M−H). Anal. Found C, 68.02; H, 7.78; N, 3.69%. ($C_{22}H_{31}NO_5$) requires C, 67.84; H, 8.02; N, 3.60%. Yield 44%.

5.7.1.11. 2-{1-[(*trans*-2-(4-Fluoro-phenyl)-cyclopropylcarbamoyl)]-cyclopentylmethyl}-4-methoxy-butyric acid, 24. δ_H ($CDCl_3$, 400 MHz) 1.01–1.16 (m, 2H), 1.40–1.52 (m, 8H), 1.81–2.00 (m, 5H), 2.47–2.54 (m, 1H), 2.78–2.82 (m, 1H), 3.28 (s, 3H), 3.33–3.43 (m, 2H), 6.05 (br s, 1H), 6.85 (d, J = 8.6 Hz, 2H), 7.09 (d, J = 8.6 Hz, 2H). LRMS: *m/z* (ES^-) 376 (M−H). HRMS: *m/z* Found 378.2077. $C_{21}H_{28}NO_4F$ requires MH^+ 378.2075. Yield 64%.

5.7.1.12. 4-Methoxy-2-{1-[2-(4-methoxy-phenyl)-ethylcarbamoyl]-cyclopentylmethyl}-butyric acid, 27. δ_H ($CDCl_3$, 400 MHz) 1.55–1.63 (m, 8H), 1.90–2.04 (m, 3H), 2.16 (dd, 1H), 2.54–2.59 (m, 1H), 2.75 (t, J = 6.8 Hz, 2H), 3.23 (s, 3H), 3.41–3.46 (m, 2H), 3.57 (t, J = 6.8 Hz, 2H), 3.85 (s, 3H), 5.84–5.89 (m, 1H), 6.85 (d, J = 8.6 Hz, 2H), 7.13 (d, J = 8.6 Hz, 2H). LRMS: (ES^-) 376 (M−H). Anal. Found C, 64.57; H, 8.09; N, 3.36%. $C_{21}H_{31}NO_5 \cdot 0.6H_2O$ requires C, 64.96; H, 8.36; N, 3.61%. Yield 43%.

5.7.1.13. 2-(1-Heptylcarbamoyl-cyclopentylmethyl)-4-methoxy-butyric acid, 28. δ_H ($CDCl_3$, 400 MHz) 0.81 (t, J = 6.7 Hz, 3H), 1.11–1.35 (m, 8H), 1.45–1.70 (m, 10H), 1.75 (d, J = 6.5 Hz, 2H), 1.85–1.95 (m, 2H), 1.95–2.14 (m, 2H), 2.40–2.50 (m, 1H), 3.15–3.25 (m, 2H), 3.30 (s, 3H), 3.47–3.53 (m, 2H), 6.23 (br s, 1H). LRMS: *m/z* (TS^+) 342 ($M+H^+$). HRMS: *m/z* Found 242.2656. $C_{19}H_{35}NO_4$ requires M^+ 342.2639. Yield 64%.

5.7.1.14. 4-Methoxy-2-[1-(3-phenyl-propylcarbamoyl)-cyclopentylmethyl]-butyric acid, 29. δ_H ($CDCl_3$, 400 MHz) 1.50–1.60 (m, 10H), 1.80–1.86 (m, 3H), 1.90–2.00 (m, 2H), 2.50–2.55 (m, 1H), 2.60 (t, J = 6.7 Hz, 2H), 3.20–3.26 (m, 4H), 3.40 (t, J = 6.7 Hz, 2H), 5.90 (br s, 1H), 7.10–7.30 (m, 5H). LRMS: *m/z* (TS^+) 362 (M+H). Anal. Found C, 69.70; H, 8.65; N, 3.86%. $C_{21}H_{31}NO_4$ requires C, 69.78; H, 8.64; N, 3.87%. Yield 68%.

5.7.1.15. 2-{1-[3-(4-Chloro-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid, 30. δ_H ($CDCl_3$, 400 MHz) 1.50–1.75 (m, 9H), 1.80–1.95 (m, 5H), 2.05–2.50 (m, 2H), 2.60 (t, $J = 6.7$ Hz, 2H), 3.25 (s, 3H), 3.20–3.30 (m, 2H), 3.35–3.40 (m, 2H), 6.10 (br s, 1H), 7.10 (d, $J = 8.6$ Hz, 2H), 7.20 (d, $J = 8.6$ Hz, 2H). LRMS: *m/z* (TS^+) 396 (M+H). HRMS: *m/z* Found 396.1943. $C_{21}H_{31}ClNO_4$ requires M^+ 396.1936. Anal. Found C, 63.73: H, 7.84; N, 3.61%. $C_{21}H_{30}NO_4Cl$ requires C, 63.71; H, 7.64; N, 3.54%. Yield 84%.

5.7.1.16. 2-{1-[3-(4-Fluoro-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid, 31. ^1H NMR (400 MHz, $CDCl_3$) 1.47–1.53 (m, 2H), 1.60–1.66 (m, 4H), 1.84 (t, $J = 6.7$ Hz, 2H), 1.90–1.94 (m, 2H), 2.00–2.03 (m, 2H), 2.40–2.43 (m, 1H), 3.24 (s, 4H), 3.38 (t, $J = 6.7$ Hz, 2H), 6.08 (br s, 1H), 6.91 (d, $J = 8.6$ Hz, 2H), 7.12 (d, $J = 8.6$ Hz, 2H), 10.45 (br s, 1H). LRMS: (ES^-) 379 (M−H). Anal. Found C, 66.35; H, 7.96; N, 3.68%. $C_{21}H_{30}FNO_4$ requires C, 66.47; H, 7.97; N, 3.69%. Yield 69%.

5.7.1.17. 4-Methoxy-2-{1-[3-(4-methoxy-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-butyric acid, 32. ^1H NMR (400 MHz, $CDCl_3$) 1.41–1.55 (m, 2H), 1.66–1.75 (m, 6H), 1.75–1.85 (m, 2H), 2.41–2.51 (m, 1H), 2.64 (t, $J = 6.7$ Hz, 2H), 3.23 (s, 3H), 3.27–3.38 (m, 2H), 3.46 (t, $J = 6.7$ Hz, 2H), 3.88 (s, 3H), 5.99 (br s, 1H), 6.89 (d, $J = 8.6$ Hz, 2H), 7.16 (d, $J = 8.6$ Hz, 2H). LRMS: (ES^-) 390 (M−H). HRMS: Found 392.2430. M+H. requires $C_{22}H_{33}NO_5$ 392.2432. Yield 84%.

5.7.1.18. 4-Methoxy-2-{1-[2-(4-methoxy-phenoxy)-ethylcarbamoyl]-cyclopentylmethyl}-butyric acid, 33. ^1H NMR (400 MHz, $CDCl_3$) δ_H 1.63–1.68 (m, 4H), 2.02–2.22 (m, 2H), 2.12–2.18 (m, 2H), 2.50–2.56 (m, 1H), 3.22 (s, 3H), 3.37 (t, $J = 6.7$ Hz, 2H), 3.68 (t, $J = 6.7$ Hz, 2H), 3.76 (s, 3H), 4.04 (t, $J = 6.7$ Hz, 2H), 6.24 (br s, 1H), 6.80–6.92 (m, 4H). LRMS: (ES^-) 392 (M−H). HRMS: Found, 394.2222. $C_{21}H_{32}NO_6$ requires M+H, 394.2225. Yield 87%.

5.8. General procedure 3. Benzyl ester deprotection

The benzyl ester (1 equiv) was dissolved in EtOH (1 M), 10 wt % Pd/C was added and the whole was shaken under a 15 psi hydrogen atmosphere for 3 h. The mixture was passed through a short plug of Arbocel to remove the catalyst and filtrate evaporated to dryness to afford the desired acids which were found to be of sufficient purity to be used with no further purification.

The following acids were prepared according to

5.8.1. General procedure 3

5.8.1.1. 2-[1-(4-Butyl-pyridin-2-ylcarbamoyl)-cyclopentylmethyl]-4-methoxy-butyric acid, 18. δ_H ($CDCl_3$, 400 MHz) 0.92 (t, 3H, Me $J = 6.9$ Hz), 1.24–2.60 (m, 19H), 3.25 (s, 3H), 3.39 (t, 2H, $J = 7.3$ Hz), 6.68–6.71 (m, 1H), 7.63–7.70 (m, 1H), 8.21 (s, 1H), 9.77 (br s, 1H). LRMS: *m/z* (ES^-) 376 (M−H). Anal. Found C, 66.20; H, 8.60; N, 7.35%. $C_{21}H_{32}N_2O_4 \cdot 0.3H_2O$ requires C, 66.05; H, 8.60; N, 7.34%. Yield 97%.

5.8.1.2. 4-Methoxy-2-(1-phenethylcarbamoyl-cyclopentylmethyl)-butyric acid, 25. δ_H ($CDCl_3$, 400 MHz) 1.42–2.08 (m, 12H), 2.43–2.57 (m, 1H), 2.83 (t, $J = 6.7$ Hz, 2H), 3.30 (s, 3H), 3.28–3.43 (m, 2H), 3.47–3.58 (m, 2H), 5.78 (br s, 1H), 7.17–7.38 (m, 5H). LRMS: *m/z* (ES^-) 346 (M−H). Anal. Found C, 69.06; H, 8.42; N, 4.01%. $C_{20}H_{29}NO_4$ requires C, 69.14; H, 8.41; N, 4.03%. Yield 68%.

5.9. 1-(2-*tert*-Butoxycarbonyl-ethyl)-cyclopentanecarboxylic acid, 3a (PG = ^{t}Bu, X = H)

A 2.5 M solution of ^{n}BuLi in hexanes (42 ml, 105 mmol) was added dropwise to a stirred solution of diisopropylamine (15 ml, 107 mmol) in 100 ml THF at −20 °C under nitrogen, and the whole stirred at this temperature for 30 min. A solution of cyclopentane carboxylic acid (5.7 g, 50 mmol) in 10 ml dry THF was then added dropwise and the resulting solution stirred at −20 °C for 30 min and then at room temperature for 2 h. The solution was then recooled to −20 °C and *tert*-butyl bromopropionate (11 g, 53 mmol) was added portionwise and the mixture then stirred at room temperature for approximately 2 h. Hundred and fifty millilitres of diethyl ether and 110 ml of 2 N HCl solution were then added in sequence, and the organic layer was separated, dried over $MgSO_4$ and evaporated to a yellow oil. This oil was taken up in diethyl ether (100 ml) and washed with saturated $NaHCO_3$ until almost all of the starting cyclopentane carboxylic acid was no longer present by TLC. The organics were then washed with 2 N HCl solution, separated, dried and evaporated in vacuo to provide 4.5 g of a yellow oil which slowly solidified on standing. This solid was recrystallised from approximately 15 ml of hexane to provide 3.49 g of the title compound as a white solid, 29%. δ_H ($CDCl_3$, 400 MHz) 1.40 (s, 9H), 1.42–1.78 (m, 6H), 1.84–1.95 (m, 2H), 2.03–2.42 (m, 4H).

5.10. General procedure 4. Preparation of acids 3

5.10.1. 1-(2-*tert*-Butoxycarbonyl-pent-4-enyl)-cyclopentanecarboxylic acid, 3b (PG = ^{t}Bu, X = allyl); 1-(2-*tert*-butoxycarbonyl-pentyl)-cyclopentanecarboxylic acid, 3c (PG = ^{t}Bu, X = *n*-propyl); 1-(2-*tert*-butoxycarbonyl-3-methoxy-propyl)-cyclopentanecarboxylic acid, 3d (PG = ^{t}Bu, X = methoxymethyl); 1-(2-*tert*-butoxycarbonyl-4-methoxy-butyl)-cyclopentanecarboxylic acid, 3e (PG = ^{t}Bu, X = methoxyethyl). 1-(2-*tert*-Butoxycarbonyl-ethyl)-cyclopentanecarboxylic acid[3,7] (**3a**, 1 equiv) was added to a stirred solution of lithium diisopropylamide (2.2 equiv) in dry THF (0.27 M total concentration) at −78 °C under nitrogen and the mixture stirred at this temperature for 45 min. A solution of the electrophile (1.1 equiv, allyl bromide, chloromethyl methyl ether or bromoethyl methyl ether) in dry THF was added dropwise over 30 min and stirring continued for a further 30 min before the reaction mixture was allowed to slowly warm to room temperature overnight. The bright orange reaction mixture was cooled to 0 °C and quenched with 2 N HCl. The solvent was removed in vacuo and the mixture extracted with EtOAc. The combined extracts were washed with brine, dried ($MgSO_4$)

and evaporated to an orange oil which was purified by flash chromatography (SiO_2; 5–10% EtOAc in pentane). The products[7] **3b**, **3d** and **3e** were isolated as pale yellow oils. Compound **3b** was taken up in EtOH at room temperature and 10 wt % Pd/C added in one portion. The mixture was shaken under a hydrogen atmosphere of 15 psi for 1 h and then filtered to remove the catalyst. The filtrate was evaporated in vacuo to a clear oil of **3c** which was used with no further purification.

Compound **3b**; PG = ^{t}Bu, X = allyl; δ_H ($CDCl_3$, 400 MHz) 1.44 (s, 9H), 1.48–1.83 (m, 6H), 2.02–2.08 (m, 2H), 2.16–2.32 (m, 3H), 2.51–2.59 (m, 1H), 2.63–2.72 (m, 1H), 4.99 (dd, 1H), 5.17 (dd, 1H), 5.76 (dddd, 1H); Anal. Found C, 68.35; H, 9.26; N, 0%. $C_{16}H_{26}O_4$ requires C, 68.06; H, 9.28; N, 0%, Yield 92%. **3c**; PG = ^{t}Bu, X = *n*-propyl; δ_H ($CDCl_3$, 300 MHz) 0.86 (t, 3H), 1.22–1.58 (m, 15H), 1.64 (m, 4H), 1.78 (dd. 1H), 2.00–2.18 (m, 3H), 2.24 (m, 1H). LRMS: *m*/*z* (TS^-) 283 (M−H), Yield 91%. **3d**; PG = ^{t}Bu, X = methoxymethyl; δ_H ($CDCl_3$, 300 MHz) 1.40 (s, 9H), 1.40–1.50 (m, 4H), 1.20–1.80 (m, 1H), 1.80–1.90 (m, 1H), 2.00 (dd, 1H), 2.00–2.05 (m, 3H), 2.20 (dd, 1H), 2.50–2.60 (m, 1H), 3.30 (s, 1H), 3.30–3.40 (m, 1H), 3.40 (t, 1H). LRMS: *m*/*z* (TS^+) 304 (MNH_4^+), Yield 66%. **3e**; PG = ^{t}Bu, X = 2-methoxyethyl; δ_H ($CDCl_3$, 400 MHz) 1.40 (s, 9H), 1.40–1.70 (m, 7H), 1.75–1.95 (m, 2H), 2.00–2.15 (m, 3H), 2.30–2.40 (m, 1H), 3.30 (s, 3H), 3.30–3.40 (m, 2H). LRMS: *m*/*z* (ES^-) 299 (M−H), Yield 55%.

5.10.2. 1-(Benzyloxycarbonyl-4-methoxy-butyl)-cyclopentanecarboxylic acid, 3f (PG = Bn, X = methoxyethyl)

5.10.2.1. Step 1. 2-(2-Methoxyethyl)malonic acid dibenzyl ester. Dibenzylmalonate (71.1 g, 0.25 mol) was added dropwise over approximately 1 h to a stirred suspension of sodium hydride (7.9 g, 0.26 mol, 80% dispersion in mineral oil) in dry THF (250 ml) under a nitrogen atmosphere at room temperature. During the addition, the temperature was allowed to rise to 40 °C. A solution of bromoethyl methyl ether (34.8 g, 0.25 mol) in THF (10 ml) was then added to the mixture at between 30 and 40 °C and the whole then stirred at reflux for 16h. The reaction mixture was cooled to room temperature and diluted with water (40 ml) and brine (40 ml) and then extracted with DCM (2× 200 ml). The combined extracts were washed with water (40 ml), dried over MgSO4 and then evaporated to provide a yellow oil. This oil was purified by silica gel chromatography using 20% ether in hexane as eluant to provide the title compound as a clear oil (58 g, 70%). δ_H ($CDCl_3$, 400 MHz) 2.01 (q, J = 7.2 Hz, 2H), 3.31 (s, 3H), 3.48 (t, J = 7.2 Hz, 1H), 3.55–3.59 (m, 2H), 5.10 (s, 4H), 7.16–7.26 (m, 10H).

5.10.2.2. Step 2. 4-Methoxy-2-methylene-butyric acid benzyl ester. The product from the above step (58 g, 0.17 mol) was taken up in dioxan (350 ml) at room temperature and a solution of KOH (10 g) in 150 ml water was added dropwise. The whole was stirred at room temperature for 16 h, and then evaporated in vacuo to approximately 100 ml volume and then 200 ml of water added. This solution was extracted with ether (3× 100 ml) to remove any unreacted starting material and the aqueous layer was then acidified to pH 1 with 2 N HCl solution. This was then extracted with ether (2× 100 ml), dried over $MgSO_4$ and evaporated in vacuo to give the title compound as a light brown oil (39 g). This oil was taken up in piperidine (2.6 ml, 0.026 mol) and dry pyridine (300 ml), and paraformaldehyde (7.1 g, 0.24 mol) added in one portion. The mixture was stirred at 60 °C for 3 h and then cooled to room temperature. The reaction mixture was poured onto ice, acidified to pH 1 with 2 N HCl and extracted with EtOAc (2× 100 ml). The combined organics were then washed with water (40 ml), saturated aq $NaHCO_3$ solution (40 ml) and then water (40 ml), and then dried over $MgSO_4$. The dried solution was then filtered and evaporated in vacuo to a clear oil. This oil was purified using silica gel chromatography and 4/1 hexane/ether as eluant to provide the title compound as a clear oil (17g, 46%). δ_H ($CDCl_3$, 400 MHz) 2.44 (t, J = 7.6 Hz, 2H), 3.46 (s, 3H), 3.62 (t, J = 7.6 Hz, 2H), 5.14 (s, 2H), 5.57 (s, 1H), 6.22 (s, 1H), 7.25–7.39 (m, 5H).

5.10.2.3. Step 3. 1-(2-Benzyloxycarbonyl-4-methoxybutyl)cyclopentane carboxylic acid, 3f. ^{n}BuLi (61.7 ml, 0.15 mol, 2.5 M solution in hexanes) was added dropwise via syringe to a stirred solution of diisopropylamine (21.4 ml, 0.15 mol) in dry THF (60 ml) under a nitrogen atmosphere at −30 °C. Once the addition was complete, the mixture was stirred at this temperature for 30 min, before adding a solution of cyclopentane carboxylic acid (8.8 g, 0.08 mol) in 30 ml dry THF. The reaction mixture was allowed to warm to room temperature over 90 min, and stirring was then continued at room temperature for a further 1 h, before cooling to −78 °C. The product from the above step (17 g, 0.08 mol) in 30 ml dry THF was added dropwise, and the mixture stirred at −78 °C for 2 h. The reaction mixture was allowed to warm to 0 °C over 2 h, and then quenched by the addition of 2 N HCl to approximately pH 2, and the reaction mixture then extracted with hexane (2× 100 ml). The combined organics were washed with water (40 ml), saturated aq $NaHCO_3$ solution (4× 40 ml) and were then dried over $MgSO_4$. Filtration and evaporation in vacuo provided a light brown oil. This oil was purified by silica gel chromatography using a gradient of hexane and EtOAc (70/30 → 50/50 → 20/80) to provide the title compound as a clear oil (8.8 g, 34%). δ_H ($CDCl_3$, 400 MHz) 1.44–2.26 (m, 14H), 2.64–2.70 (M, 1H), 3.29 (s, 3H), 3.42 (t, J = 6.7 Hz, 2H), 5.10 (s, 2H), 7.04–7.23 (m, 5H).

5.11. General procedure 5. Preparation of cyclopropylamines 7

5.11.1. *trans*-2-(4-Chlorophenyl)-cyclopropylamine, 7a (R = Cl); *trans*-2-(4-methoxyphenyl)-cyclopropylamine, 7b (R = OMe); *trans*-2-(4-fluorophenyl)-cyclopropylamine, 7c (R = F)

5.11.1.1. Step 1. Cyclopropanation. A mixture of the requisite styrene **4** (1 equiv) and rhodium acetate dimer (0.05 equiv) in toluene (2 M) was heated to 85 °C before the addition of ethyl diazoacetate (1 equiv) over 30 min and the whole was then heated at 80 °C for a further 1 h.

The solution was concentrated in vacuo, and the residue purified by flash column chromatography using DCM as eluant to provide the cyclopropane carboxylate esters **5** as oils.

The following cyclopropanes were prepared according to Step 1;

5.11.2. *trans*-2-(4-Chlorophenyl)-cyclopropane carboxylic acid ethyl ester, 5a; R = Cl. δ_H ($CDCl_3$, 400 MHz) 1.15–1.30 (m, 4H), 1.50–1.70 (m, 1H), 1.80–1.90 (m, 1H), 2.40–3.55 (m, 1H), 4.20 (q, J = 7.2 Hz, 2H), 6.95 (d, J = 8.6 Hz, 2H), 7.24 (d, J = 8.6 Hz, 2H). LRMS: m/z (TS^+) 242 ($M+NH_4^+$). Yield 37%.

5.11.3. *trans*-2-(4-Methoxyphenyl)-cyclopropane carboxylic acid ethyl ester, 5b; R = OMe. δ_H ($CDCl_3$, 400 MHz) 1.20–1.38 (m, 5H), 1.83 (dd, J = 16.0, 7.8 Hz, 1H), 2.50 (dd, J = 16.0, 9.3 Hz, 1H), 3.80 (s, 3H), 4.16 (q, J = 7.2 Hz, 2H), 6.82 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.6 Hz, 2H). Yield 31%.

5.11.4. *trans*-2-(4-Fluorophenyl)-cyclopropane carboxylic acid ethyl ester, 5c; R = F. δ_H ($CDCl_3$, 400 MHz) 1.07 (t, J = 7.2 Hz, 3H), 1.30–1.39 (m, 1H), 1.74–1.78 (m, 1H), 2.13 (dd, J = 16.0, 7.8 Hz, 1H), 2.45 (dd, J = 16.0, 7.8 Hz, 1H), 3.86 (q, J = 7.2 Hz, 2H), 6.82 (d, J = 8.4 Hz, 2H), 7.03 (d, J = 8.4 Hz, 2H). Yield 41%.

5.11.4.1. Step 2. Formation of *trans*-cyclopropane carboxylic acids. The carboxylate esters **5** from Step 1 (1 equiv) were dissolved in ethanol (2 M) at room temperature under a nitrogen atmosphere, and sodium methoxide (4 equiv) was added portionwise over 15 min. After the addition was complete the combined mixture was refluxed for 18h. The solution was concentrated in vacuo and the resulting residue diluted with DCM (100 ml) and water (50 ml). The organic layer was removed, and the aqueous layer re-extracted with DCM (2×50 ml). The combined organics were dried over $MgSO_4$ and then evaporated to provide a clear oil. Acidification of the aqueous layers with concd HCl to pH 1 resulted in a white precipitate, which was filtered and dried under vacuum to provide a white powder. The oil was taken up in methanol (50 ml) and water (50 ml) and solid LiOH (1 equiv) added. The resulting clear solution was then heated at 70 °C overnight. The reaction mixture was cooled, concentrated in vacuo and acidified with concd HCl to pH 1. The resulting white precipitate was extracted with EtOAc (3× 50 ml), and the combined organic extracts were dried with $MgSO_4$ and then evaporated to dryness and combined with the white solid from above, to provide the *trans* acids **6**.

The following cyclopropanes were prepared according to Step 2;

5.11.5. *trans*-2-(4-Chlorophenyl)-cyclopropane carboxylic acid, 6a; R = Cl. δ_H ($CDCl_3$, 400 MHz) 1.30–1.40 (m, 1H), 1.60–1.70 (m, 1H), 1.80–1.90 (m, 1H), 2.50–2.60 (m, 1H), 7.00 (d, J = 8.4 Hz, 2H), 7.26 (d, J = 8.4 Hz, 2H). LRMS: m/z (ES^-) 195 (M−H). Yield 96%.

5.11.6. *trans*-2-(4-Methoxyphenyl)-cyclopropane carboxylic acid, 6b; R = OMe. δ_H ($CDCl_3$, 400 MHz) 1.30–1.41 (m, 1H), 1.58–1.69 (m, 1H), 1.79–1.90 (m, 1H), 2.53–2.62 (m, 1H), 6.83 (d, J = 8.4 Hz, 2H), 7.04 (d, J = 8.4 Hz, 2H). Yield 78%.

5.11.7. *trans*-2-(4-Fluorophenyl)-cyclopropane carboxylic acid, 6c: R = F. δ_H ($CDCl_3$, 400 MHz) 1.40–1.46 (m, 1H), 1.78–1.85 (m, 1H), 2.57 (dd, J = 16.0, 7.8 Hz, 1H), 2.83 (dd, J = 16.0, 7.8 Hz, 1H), 6.77 (d, J = 8.4 Hz, 2H), 7.16 (d, J = 8.4 Hz, 2H). Yield 78%.

5.11.7.1. Step 3. Curtius rearrangement. The products **6** from Step 2 (1 equiv), DPPA (1.1 equiv) and triethylamine (1.5 equiv) were combined in *tert*-butanol (0.5 M) under nitrogen and heated at 90 °C for 48 h before cooling to room temperature. The mixture was diluted with EtOAc (20 ml) and saturated Na_2CO_3 solution (20 ml) and the organic layer separated. The aqueous layer was re-extracted with EtOAc (20 ml) and the combined organic layers were dried over $MgSO_4$, filtered and evaporated. The resulting residue was purified by flash chromatography using 20% EtOAc in pentane as eluant to provide the intermediate *tert*-butyl carbamates as white solids. The carbamates were then taken up in EtOAc (0.2 M), cooled to 0 °C and hydrogen chloride gas was bubbled through the solution for 30 min. The solution was then concentrated in vacuo to give a pale yellow solid of the amine HCl salt **7**.

The following cyclopropanes were prepared according to Step 3;

5.11.8. *trans*-2-(4-Chlorophenyl)-cyclopropylamine, 7a; R = Cl. δ_H ($CDCl_3$, 400 MHz) 1.30–1.40 (m, 1H), 1.40–1.50 (m, 1H), 2.30–2.40 (m, 1H), 2.80–2.90 (m, 1H), 7.15 (d, J = 8.6 Hz, 2H), 7.30 (d, J = 8.6Hz, 2H). Yield 81%.

5.11.9. *trans*-2-(4-Methoxyphenyl)-cyclopropylamine, 7b; R = OMe. δ_H ($CDCl_3$, 400 MHz) 0.87–1.04 (m, 2H), 1.79–1.90 (m, 1H), 2.43–2.54 (m, 1H), 3.80 (s, 3H), 6.80 (d, J = 8.6Hz, 2H), 6.98 (d, J = 8.6Hz, 2H). Yield 89%.

5.11.10. *trans*-2-(4-Fluorophenyl)-cyclopropylamine, 7c; R = F. δ_H ($CDCl_3$, 400 MHz) 0.93–0.97 (m, 2H), 2.03 (dd, J = 16.0, 7.8 Hz, 1H), 2.05 (dd, J = 16.0, 7.8 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 8.6 Hz, 2H). Yield 80%.

5.12. General procedure 6. Preparation of phenpropylamines 9

5.12.1. 3-(4-Methoxy-phenyl)-propylamine, 9a (R = OMe); 3-(4-fluoro-phenyl)-propylamine, 9b (R = F). A solution of the halide **8** (1 equiv), acrylonitrile (1.1 equiv), tri-*o*-tolylphosphine (0.1 equiv), palladium acetate (0.1 equiv) and triethylamine (3 equiv) in acetonitrile (0.2 M) was refluxed under nitrogen for 14 h. The reaction mixture was diluted with EtOAc (50 ml), and washed with 2 M $NaHCO_3$ (100 ml), and the organic layer was dried over $MgSO_4$ and filtered. The filtrate was evaporated in

vacuo and the residue purified by column chromatography using 5% EtOAc in pentane as eluant to provide the diastereomeric vinyl nitriles, usually favouring the *trans* isomer. The products from this step (1 equiv) were taken up in ammonium hydroxide solution and ethanol (1:1 mixture, to ca. 0.15 M) and the mixture was shaken under 40 psi hydrogen pressure with Raney Ni (20 wt %) for 12 h. The reaction mixture was filtered through Arbocel and washed with ethanol (20 ml). The filtrate was evaporated in vacuo to give the amines **9** which were pure enough to be used with no further purification.

The following amines were prepared according to

5.12.2. General procedure 6

5.12.2.1. 3-(4-Methoxy-phenyl)-propylamine, 9a; R = OMe. ^{1}H NMR (400 MHz, $CDCl_3$) 1.70–1.76 (m, 2H), 2.06 (br s, 2H), 2.52 (t, J = 7.4Hz, 2H), 2.68–2.74 (m, 2H), 3.78 (s, 3H), 6.78 (d, 2H), 7.03 (d, 2H). LRMS: (TS^+) 376 (M+H). Yield 43%.

5.12.2.2. 3-(4-Fluoro-phenyl)-propylamine, 9b; R = F. ^{1}H NMR (400 MHz, $CDCl_3$) 1.70–1.78 (m, 2H), 2.25 (br s, 2H), 2.47–2.50 (t, 2H), 2.64 (br s, 2H), 6.97 (d, J = 8.6Hz, 2H), 7.20 (d, J = 8.6Hz, 2H). LRMS: (TS^+) 364 (M+H). Yield 88%.

5.13. 2-(4-Methoxy-phenoxy)-ethyl amine, 13

Triphenylphosphine (1.78 g, 6.8 mmol) was dissolved in 20 ml DCM at room temperature under nitrogen and first DEAD (1.18 ml, 6.8 mmol), then ethyl hydroxy phthalimide (1 g, 5.2 mmol) were added portionwise. The orange solution was stirred for 4 h, and the solvents then removed under vacuum. The resulting orange residue was purified by column chromatography using DCM as eluant to provide the phenoxy compound as an orange solid (768 mg, 50%). This was taken up in methanol (5 ml), hydrazine hydrate added (150 μl, 3.1 mmol) in one portion and the whole refluxed for 4 h. A white precipitate was formed upon cooling, and the suspension was stirred at room temperature for 48 h. The methanol was removed in vacuo, and the residue partitioned between EtOAc (50 ml) and citric acid (50 ml), and the layers separated. The aqueous layer was basified to pH 8 with 1 N NaOH and extracted with EtOAc (2× 20 ml). The organic layers were combined, dried ($MgSO_4$) and evaporated to give a colourless oil of the title amine **13** (270 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.72 (br s, 2H), 3.02 (t, J = 7.2 Hz, 2H), 3.76 (s, 3H), 3.95 (t, J = 7.2 Hz, 2H), 6.80–6.90 (m, 4H). LRMS: m/z (TS^+) 168 (M+H).

5.14. 4-Methoxy-2-[1-(1-methyl-2-phenyl-ethylcarbamoyl)-cyclopentylmethyl]-butyric acid, 26

The benzyl ester **10** (473 mg, 1.18 mmol) was taken up in ethanol (10 ml) at room temperature and hydrogenated at 60 psi hydrogen pressure over 10 wt % Pd/C for 16 h. The reaction mixture was filtered through a short plug of Arbocel and washed with ethanol. The combined filtrates were evaporated in vacuo to give a colourless oil, which was purified by column chromatography using 0%, then 1, then 2, then 5% methanol in DCM to provide the ring opened phenethylamine **26** as a clear oil (319 mg, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 1.12 (d, 3H J = 6.5 Hz), 1.53–2.00 (m, 13H), 2.47–2.52 (m, 1H), 2.78–2.83 (m, 2H), 3.31–3.38 (m, 3H), 3.42–3.46 (m, 2H), 4.20–4.23 (m, 1H), 5.58–5.63 (m, 1H), 7.10–7.15 (m, 3H), 7.20–7.24 (m, 2H). LRMS: (ES^-) 362 (M−H). Anal. Found C, 65.87; H, 8.40; N, 3.61%. $C_{21}H_{29}NO_4$·0.3DCM requires C, 66.11; H, 8.23; N, 3.62%.

5.15. General procedure 7. Classical resolution of acids 3

The racemic acid **3** (1 equiv) and (+)-pseudoephedrine (1 equiv) were dissolved in hexane (which may require heating with a heat gun). The solution was allowed to cool to room temperature overnight, by which time white crystals had appeared. These were filtered off and re-subjected to the crystallisation procedure in the minimum quantity of hexane. Typically 9–10 recrystallisations were required to obtain material of >90% ee. Optical purity at this stage could be estimated from the ^{1}H NMR shifts of the diastereomeric pseudoephedrine salts in $CDCl_3$—the *tert*-butyl ester peaks typically showed some separation, and the relative abundance of each peak gave a good estimate of enantiomeric excess. The salt was then taken up in EtOAc, washed with 0.5 M HCl (aq) and the organic layer dried over $MgSO_4$, filtered and evaporated in vacuo to provide the ***R***-alkyl versions of **3b–c**, and the *S*-alkoxyethyl versions of **3d–e**, in typically 30% yield. These acids were then coupled to the requisite amine according to Scheme 1. Examples of non-racemic material synthesised according to

5.15.1. General procedure 7

5.15.1.1. *R*-2, *R*-2-[1-(5-Ethyl-[1,3,4]thiadiazol-2-yl-carbamoyl)-cyclopentylmethyl]-pentanoic acid. $[\alpha]_D$ −9.0 (c 0.1, MeOH), Anal. Found C, 56.46; H, 7.46; N, 12.37%. ($C_{16}H_{25}N_3O_3S$) requires C, 56.62; H, 7.44; N, 12.37%.

5.15.1.2. *S*-30, 2-{1-[3-(4-Chloro-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid. $[\alpha]_D$ (for Na salt) −0.06 (c 3.2, MeOH), Anal. Found C, 63.73: H, 7.84; N, 3.61%. $C_{21}H_{30}NO_4Cl$ requires C, 63.71; H, 7.64; N, 3.54%.

5.15.1.3. *S*-31, 2-{1-[3-(4-Fluoro-phenyl)-propylcarbamoyl]-cyclopentylmethyl}-4-methoxy-butyric acid. $[\alpha]_D$ −0.01 (c 1.87, EtOH), HRMS: m/z Found 380.2232; $C_{21}H_{31}NO_4F$ requires MH^+ 380.2225; Anal. Found C, 66.73: H, 8.04; N, 4.11%. $C_{21}H_{30}NO_4F$ requires C, 66.47; H, 7.97; N, 3.69%.

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