Design and Synthesis of Thrombin Inhibitors: Analogues of MD-805 with Reduced Stereogenicity and Improved Potency

Derek Brundish,[†] Alice Bull,[†] Vera Donovan,[†] Joseph D. Fullerton,[†] Sheila M. Garman,[†] Judy F. Hayler,[†] Diana Janus,[†] Peter D. Kane,*[,†] Mark McDonnell,[†] Garrick P. Smith,[†] Robert Wakeford,[†] Clive V. Walker,[†] Graham Howarth,[||] William Hoyle,[||] Mark C. Allen,[§] John Ambler,[‡] Keith Butler,[‡] and Mark D. Talbot[‡]

Department of Chemistry, Department of Biology, and Drug Discovery Support, Novartis Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex RH12 5AB, U.K., and Ciba Central Research Laboratories, Hulley Road, Macclesfield, Cheshire SK10 2NX, U.K.

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Mitsubishi's MD-805, a potent and selective inhibitor of thrombin which contains four stereogenic centers, has been the starting point for an optimization program. A systematic synthetic study resulted in thrombin inhibitors achiral at P2 and P3 but with a 10-fold increase in potency over the original lead. A number of 4-substituted piperidines were synthesized and examined as replacements for 2-carboxy-4-methylpiperidine at P2; 4-fluoroethylpiperidine (FEP) among others provided inhibitors (e.g. **45g**) of increased potency. An enantioselective route was developed to 3(*R*)-methyl-1,2,3,4-tetrahydroquinolinesulfonyl chloride. Inhibitors containing this enantiomerically pure P3 **(42d)** had similar potency to the racemic material and provided support, with modeling studies, for the preparation of the gem 3,3-disubstituted compounds. A series of inhibitors containing the novel 3,3-dimethyl-1,2,3,4-tetrahydroquinolinesulfonyl (DMTHQS) P3 (Table 5) were synthesized and showed a similar activity profile as the monomethyl series. The combination of P3-DMTHQS, P2-FEP, and P1-arginine **(45g)** had a K_i of 6 nM (MD-805 K_i = 85 nM). In animal models of both venous and arterial thrombosis, one inhibitor (**42e)** was shown to produce a dose-dependent inhibition of thrombus formation that in some situations was superior to that of MD-805.

Introduction

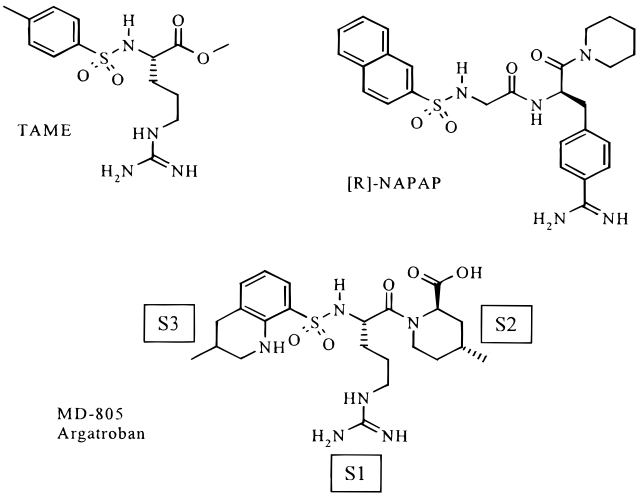
Over the past 15 years considerable effort has been made to discover direct inhibitors of thrombin.[1−4] While a number of purely parenteral agents, e.g. Hirudin and hirulog-8,[1] have been developed, the main emphasis within pharmaceutical research has been to develop low-molecular-weight thrombin inhibitors[2] with the potential for oral delivery.

Of the low-molecular-weight inhibitors that have been prepared, the majority of those reported derive in some way from the synthetic thrombin substrate tosylarginine methyl ester (TAME) (Chart 1).[5] Early research by various groups had led independently to three different inhibitor types: MD-805 (Argatroban)[6] identified by Okamoto and co-workers,[7−11] subsequently launched as Novastan in Japan by Mitsubishi and being developed in the United States by Texas Biotechnology; NAPAP developed by Sturzebecher and co-workers;[12,13] GYKI-14166, a tripeptide aldehyde originally cited by Richter,[14] a modified version of which, D-MePhe-Pro-Arg-H (Efegatran),[15,16] is in clinical trials. These structures have been further developed and refined in attempts to develop drugs with increased efficacy and bioavailability.[1,2]

Such compounds as GYKI-14166 derive appreciable potency by virtue of an electrophilic aldehyde group which forms a reversible hemiketal with the catalytic triad serine of thrombin. Others have also utilized aldehydes,[17] and a number of compounds have been reported where this type of interaction has been further exploited by introducing alternative electrophilic groups, e.g. trifluoromethyl ketones,[18] boronic acids and esters.[19] The selectivity of such inhibitors is therefore largely governed by efficient occupancy of the unique space and interaction with structural features in the vicinity of the catalytic triad; in the case of thrombin the lipophilic pockets defined by the insertion loop are important.

With the above considerations in mind, we had initiated a thrombin inhibitor program using Misub-

Chart 1. Structures of MD-805, NAPAP, and TAME



* Address for correspondence: Tripos Receptor Research Ltd., Bude-Stratton Business Park, Bude, Cornwall EX23 8LY, U.K. E-mail: pkane@tripos.com.
[†] Department of Chemistry.
[‡] Department of Biology.
[§] Drug Discovery Support.
[||] Ciba Central Research Laboratories.

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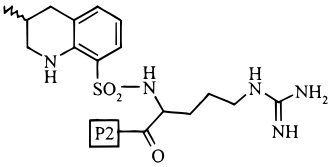
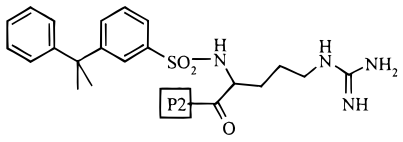
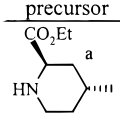
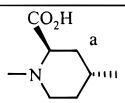
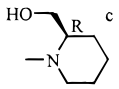
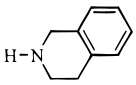
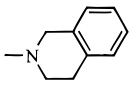
Table 1. Comparison of 3-Methyltetrahydroquinolylsulfonyl (MTHQS) and *m*-Cumylphenylsulfonyl (mCPS) at the P3 Position





amine precursor	coupling method	MTHQS inhibitor	K_i (μM)[d]	P2	mCPS inhibitor	K_i (μM)[d]
	2	Argatroban	0.061 0.085[e]		**48a**	1.2
16b	2	**47a**	0.023		**44**	3
15	2 (1[b])	**47b**	0.08		**38a**	2.9
	1	**39a**	0.795		**38b**	0.11
	1	**39b**	0.023		**38c**	0.049

[a] Synthesis as described by Mitsubishi. [b] Coupling method 1 used for mCPS-Arg-prolinol. [c] (*S*)-Isomer also prepared from piperidine **6a** (K_i = 6 μM). [d] Figures quoted are for bovine thrombin unless otherwise stated. [e] Against human thrombin.

ishi's MD-805 as the starting point, a non-transition-state reversible inhibitor with good potency and selectivity for thrombin. The decision was taken at an early stage to avoid the use of strongly electrophilic or potentially reactive groups as an aid to achieving potency; it was considered that this could be detrimental in terms of selectivity, oral bioavailability, and toxicity. The ultimate goal was to develop both parenterally and orally active thrombin inhibitors, and this article details our initial work in which arginine was conserved in the P1 position.

Initial Considerations

The compound MD-805, which inhibits bovine and human thrombin with a reported K_i of 19 and 39 nM, respectively,[6] was the culmination of a large investigation by Mitsubishi.[20] The structure−activity relationship of MD-805 can be considered in three parts: the central amino acid arginine (P1) which occupies the thrombin specificity pocket and interacts with aspartic-189, the C-terminal piperidine amide (P2), and the N-terminal methyltetrahydroquinolinesulfonyl (MTHQS) group (P3). The C- and N-terminal groups both occupy lipophilic pockets, created to a large extent by the presence of the thrombin-60 insertion loop.

The presence of chirality within a molecule can make its progression as a drug candidate difficult, mainly because of chemical and clinical development and regulatory issues. Individual stereoisomers can often have differing pharmacological, pharmacokinetic, and toxicological profiles and the future progression of racemic mixtures is likely to require the individual evaluation of constituent isomers. MD-805 has four stereogenic centers: the compound is a racemic mixture of isomers with respect to the methyl group in the THQS ring; the arginine is of the natural L-configuration; the piperidine is disubstituted and is of the 2*R*,4*R* configuration. The above considerations led us in the first instance to investigate the simplification of the MD-805 structure with respect to chirality.

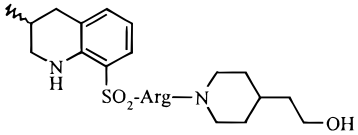
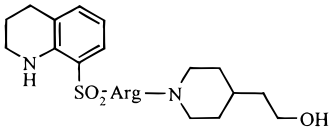
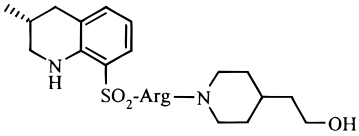
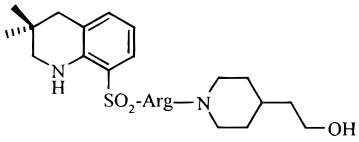
Strategy and Results

P3 Studies. Formally thrombin has no S3 subsite, as the natural substrate fibrinogen possesses a glycine in what would be the P3 position. However in the substrate/thrombin model the pocket proximal to the P3 glycine is occupied by the lipophilic side chains of P8-Leu and P9-Phe of fibrinogen, which loop around to make this interaction.[21−23] This subsite is accessible from P3 if a D-side chain is present (e.g. D-Phe). In the MD-805/thrombin complex the MTHQS ring is located in this pocket, termed P3* by Banner;[23] for convenience we refer to this part of the inhibitor as P3.

A large number of simpler nonchiral aryl alternatives to MTHQS were investigated, but the majority of these showed little activity. The Mitsubishi group had previously discovered that the phenylsulfonyl group, substituted at the 3-position with various groups, i.e., alkyl, alkoxy, and phenylalkyl, exhibited activity when incorporated at the P3 position of inhibitors;[20] this finding led us to look at *m*-cumylphenylsulfonyl (mCPS) as a possible P3 (Table 1). Compounds incorporating this group displayed significant inhibitory activity, in some cases superior to that of MTHQS, but generally MTHQS compounds were of greater potency, particularly it seemed when the P2 piperidide or pyrrolidine bore a polar substituent (Table 1). For compounds where P2 was a simple ring system (e.g. **38b**, **38c)** the differences were less marked.

That the 3-methyl group of MTHQS makes good interactions in the aryl binding S3 site[21] and contributes significantly to potency is confirmed by comparison of **42d** and **42e** (Table 2), which differ only by the methyl group and yet have an order of magnitude potency

Table 2. Comparison of 3-Substituted Tetrahydroquinolines in the P3 Position

Inhibitor	amineP2 precursor	coupling method	P3	K_i (μM)[a]
42b	**17b**	2		0.052 0.044[b]
42c	**17b**	2		0.49
42d	**17b**	2		0.046
42e	**17b**	2		0.048 0.032[b]

[a] All quoted K_i's are for bovine thrombin unless other stated. [b] For human thrombin.

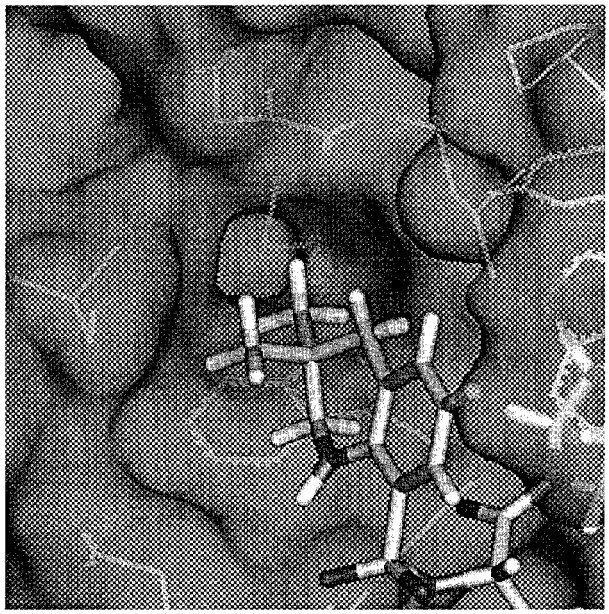


Figure 1. *gem*-Dimethyl interaction with the S3 pocket of thrombin.

difference. Previous reports concerning the profiling of individual isomers indicate a 3-fold difference in activity between the 3*R*- and 3*S*-methyl compounds,[24] but with certain types of inhibitors we observed the in vitro activity differences of the isomers to be negligible (e.g. **42b** and **42d**). Modeling studies carried out with both the 3*R* and 3*S* isomers, and also the 3,3-*gem*-dimethyl compound, indicated all to have a reasonable fit and there to be a considerable amount of unoccupied space in this region of thrombin (Figure 1). This degree of space is further confirmed by examination of crystal structures of other types of inhibitors, i.e. NAPAP and PPACK bound to thrombin.[13]

A good rationale therefore existed to remove the stereogenicity at the 3-position by synthesizing the *gem*-dimethyl-substituted THQS compound. It was reasoned that inhibitors containing this moiety should be at least equally potent to their monomethyl counterparts. We therefore undertook the synthesis of the required dimethyltetrahydoquinolylsulfonyl chloride (DMTHQSCl) (Scheme 3) and evaluation of inhibitors containing this P3.

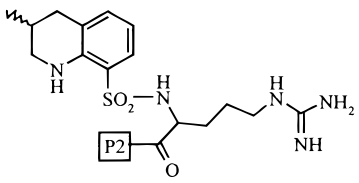
The results detailed in Table 2 confirmed our supposition to have been correct, the 3,3-dimethylquinolyl compound **42e**, the racemic mixture **42b**, and the 3*R* compound **42d** all had similar activity. To further test the extent of this lipophilic cleft, the *gem*-3,3-diethyl compound was also prepared and showed good potency (K_i = ~20 nM).

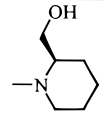
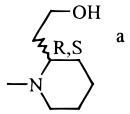
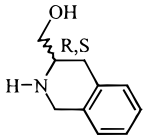
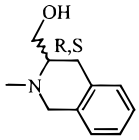
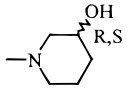
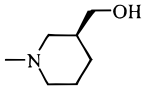
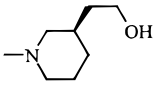
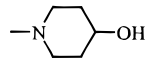
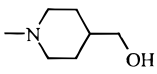
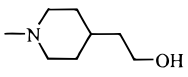
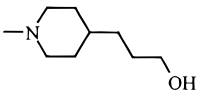
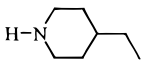
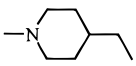
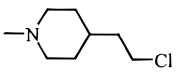
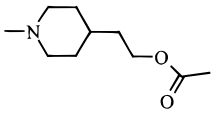
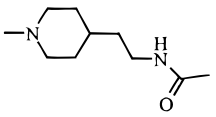
P2 Studies. A comparison of the X-ray crystallography structure of the thrombin/inhibitor complex with the binding mode of the natural substrate fibrinogen reveals that MD-805 interacts in a non-substrate-like way,[13] with the piperidine moiety located in the thrombin S2 subsite (Berger and Schechter notation) and hence conveniently termed P2.

MD-805 contains a disubstituted piperidide in the P2 position, and the diastereomer with the 2*R*,4*R* configuration is the most potent.[7] The presence of the carboxylic acid in the 2-position appears to be largely to provide an internal counterion for the arginine, as reducing the basicity is considered important for increasing the tolerability of this class of compound.[10] However it has also been speculated that the carboxyl anion interacts with some part of thrombin and contributes to potency.[25] The presence of the carboxyl group also has the undesirable effect of conferring two chiral centers on P2. It had been ably demonstrated by Mitsubishi that piperidide is favored for the P2 position, but we sought to find simpler piperidides that would provide potent and selective thrombin inhibitors.

A detailed exploration of different positions on the piperidide nucleus confirmed both the 2- and 4-positions to be available for substitution (Table 3). However from modeling the 4-position appeared to have greater scope

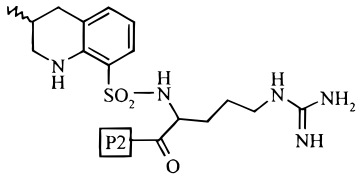
Table 3. In Vitro Inhibitory Activities of MTHQS-Arg-Piperidide Compounds Containing Piperidide Substituted with Alkyl, Hydroxyalkyl, Acetoxy, and Acetamido at the 3-Position

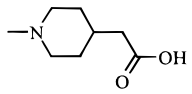
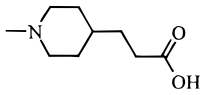
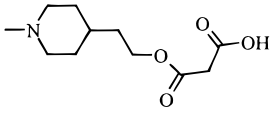
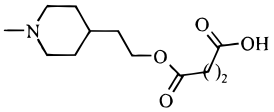
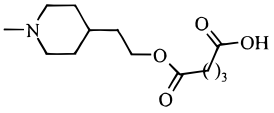
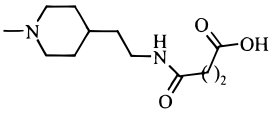
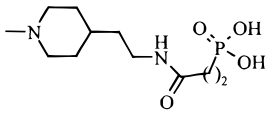
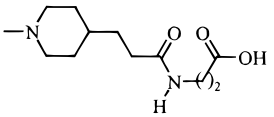
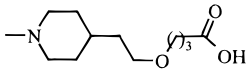
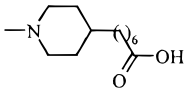


Inhibitor	piperidine precursor	coupling method	P2	K_i (μM)[b]
47a	**16b**	2		0.023
47c	**16c**	2	 [a]	0.064
39c		1		0.89
47d	**16d**	2		0.36
47e	**16e**	2		1.5
47f	**16f**	2		1.35
47g	**16g**	2		5.18
42a	**17a**	2		0.064
42b	**17b**	2		0.052 0.044[c] (IC_{200} = 1.65)[d]
47h	**16i**	2		0.185
39d		2		0.027 (IC_{200} = 6.27)[d]
45a	**22b**	2		0.019 0.011[c] (IC_{200} = 2.7)[d]
45b	**16h**	2		0.015
45c	**27a**	2		0.045 0.066[c] (IC_{200} = 1.8)[d]

[a] Separate isomers isolated. [b] Quoted K_i's are against bovine thrombin unless otherwise stated. [c] K_i against human thrombin. [d] The dose in μM to double the control plasma APTT value in human plasma.

Table 4. In Vitro Inhibitory Activities of MTHQS-Arg Compounds with Piperidine Carboxylic Acid at P2



Inhibitor	piperidine	method	P2	K_i (μM)[a]
48b	**28**	2		2.6
48c	**30**	2		18.4
43a	**-**	2		0.36
45d	**9b**	2		0.034
43b	**-**	2		0.17
48d	**27b**	2		0.65
50	**27c**	2		35.2
49	**30**	2		0.31 0.28[b]
48e	**21**	2		0.98
48f	**34**	2		1.98

[a] Quoted K_i's for bovine thrombin unless otherwise stated. [b] Against human thrombin.

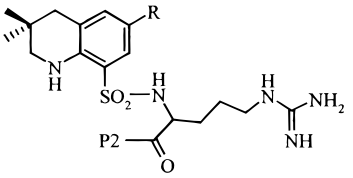
for substitution and involved nonchiral building blocks; as a consequence of this we concentrated on a more extensive exploration of 4-substituted piperidides.

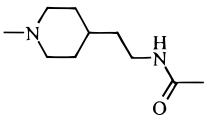
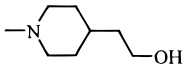
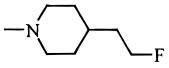
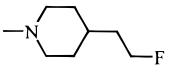
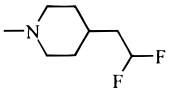
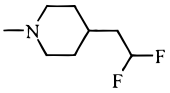
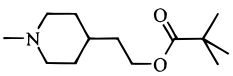
It is interesting to note that while the 4-ethyl **39d** and 4-chloroethylpiperidyl **45a** compounds are superior in absolute potency to the 4-hydroxyethyl **42b** and 4-acetamidopiperidyl **45c** inhibitors, they are less effective in the more relevant plasma-based APTT assay. This is no doubt in part due the increased nonspecific protein binding which results from their greater lipophilic character.

We were interested to investigate the limits of substitutions in the piperidine 4-position and to see if we, like Mitsubishi, could introduce acidic functions as potentially toxicological modifying moieties. Such 4-substituted piperides as those contained in **42b**, **45b**, and **45c** were seen as ideal scaffolds upon which to introduce such acidic groups. Table 4 shows some of the compounds which were prepared with this in mind; as can be seen the activities which were obtained were generally disappointing. The succinate ester **45d** alone approached the activity of the parent structures, a compound with doubtful in vivo stability.

A 4-alkyl-substituted piperidide is effective at P2 because of the good van der Waals contacts that are made with the largely lipophilic pocket defined by the 60 insertion loop, in this area Trp-60 being the most important residue. A closer examination of the structure—

Table 5. In Vitro Inhibitory Activities of Compounds Containing 3,3-Dimethyltetrahydroquinoline at P3



Inhibitor	piperidine precursor	R	method	P2	K_i[a] (μM)	IC_{200}[b] (μM)
45e	**27a**	H	2		0.043	2.15
42e	**17b**	H	2		0.032	1.9
45f	**22a**	Cl	2		0.0066	3.25
45g	**22a**	H	2		0.0057	2.1
45h	**24**	Cl	2		0.0121	6
45i	**24**	H	2		0.006	1.5
45j	**19a**	H	2		0.056	8.5

[a] All K_i's quoted are for human thrombin. [b] The dose to double the control plasma APTT value in human plasma.

activity relationship for the various P2's that we had prepared led us to speculate that an ion−dipole interaction between an electronegative group on the inhibitor and the proximal protonated lysine-60 of the loop might be important. This would provide one explanation for the good potencies of inhibitors such as **42b**, acetate **45b**, and acetamide **45c**. A counter argument would be that the lipophilic part of the molecule was providing the majority of the binding energy through good van der Waals contact and that the pendant polar groups were merely tolerated; this is born out to some extent by the observation that the 4-ethylpiperidide compound **39d** has good potency.

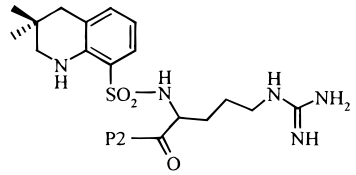
A series of compounds were prepared which combined the newly discovered DMTHQS P3 with a number of reasonably simple P2's (Table 5); the compounds containing fluoro-substituted ethylpiperidides stand out. Compounds **45g** and **45i** were the most potent that we had seen in this arginine P1 series, with K_i's an order of magnitude lower than that of MD-805. As fluorine increases the log *P* and has a high electronegativity, this finding could be explained in terms of either of the previously argued modes of interaction vide supra.

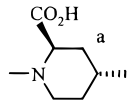
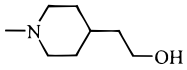
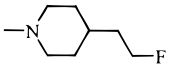
In our hands all the DMTHQS compounds in Table 5 showed superior in vitro potency to MD-805 (K_i = 85 nM) and comparable selectivity profiles, as shown for **42e** and **45h** (Table 6). However when comparing IC_{200} values MD-805 was found to be the more potent. If one looks within the series of DMTHQS compounds, their effectiveness within plasma tends to deteriorate in a trend that parallels the increasing lipophilicity of substituents. This effect is particularly apparent when comparing for instance **42e** and **45h**; the former contains hydroxyethyl in P2, and while it is approximately 3-fold less active than **45h** in vitro it is 3-fold more active than the difluoro- and chloro-containing **45h** in plasma. **45j** is another example of a lipophilic compound with reasonable potency but with a reduced plasma effect. A possible explanation for this is that the compounds with higher lipohilicity are likely to be more protein-bound thus reducing their effectiveness in plasma.

The effects of **42e** and MD-805 on venous thrombosis were determined in an acute model in the rat[26] and the effects on arterial thrombosis in an acute model of injury-induced thrombus formation in the rat dorsal aorta.[27] Both intravenous and subcutaneous routes of administration were examined.

In the venous thrombosis model MD-805 was approximately 3-fold more potent than **42e** by the iv route, but interestingly the situation was reversed when subcutaneous administration was used (Table 7). In the

Table 6. Selectivity Profile for **42e**, **45g**, and MD-805



P2	Thrombin	Plasmin	K_i (μM) Trypsin[a]	Chymotrypsin[a]	IC_{200} (μM)[b]
 (**MD805**)	0.085	U98[c]	22.8	U196	0.75
 (**42e**)	0.032	210	7.1	U83	1.9
 (**45g**)	0.0057	N/A[d]	25.6	U25.6	2.1

[a] Values shown are for bovine trypsin and chymotrypsin; all other values are for the human enzyme. [b] The dose to double the control plasma APTT value in human plasma. [c] U denotes inactive at stated concentration. [d] Not available.

Table 7. Effect of **42e** and MD-805 on Venous Stasis Thrombosis in the Rat

dose[a] (mg/kg)	percentage inhibition[b] **42e** iv	**42e** sc	MD-806 iv	MD-806 sc
6.0		95.2 ± 4.7	89.5 ± 1.4	100.0 ± 0.0
3.0		71.7 ± 12.9	81.3 ± 3.8	80.3 ± 6.8
2.0		79.8 ± 12.9	13.3 ± 30.2	
1.7			7.8 ± 18.6	32.4 ± 11.0
1.0	100.0 ± 0.0	50.0 ± 10.1		25.4 ± 11.9
0.3	92.2 ± 5.5	2.3± 6.1		9.9 ± 12.8
0.1	45.1 ± 7.2		100.0 ± 0.0	
0.05	2.3 ± 15.1			
0.03			55.8 ± 15.1	
0.01			13.3 ± 18.3	

[a] The compounds were administered either 2 min (intravenously) or 1 h (subcutaneously) prior to induction of thrombus formation. [b] Results are expressed as percentage inhibition of the mean control thrombus weight ± standard error of the mean ($n = 5$).

Table 8. Effect of Intravenous Administration of **42e** and MD-805 on the Platelet and Fibrin(ogen) Accumulation in the Rat Model of Arterial Thrombosis

dose[a] (mg/kg)	percentage inhibition[b] **42e** platelets	**42e** fibrinogen	MD-805 platelets	MD-805 fibrinogen
3.0	94.2 ± 1.4	82.0 ± 4.1	89.5 ± 1.4	77.1 ± 4.1
2.0			81.3 ± 3.8	67.8 ± 6.2
1.0	61.3 ± 10.1	52.9 ± 7.5	13.3 ± 30.2	37.4 ± 15.6
0.3	58.0 ± 9.3	36.4 ± 11.3	7.8 ± 18.6	24.2 ± 13.0
0.1	4.4 ± 18.9	31.5 ± 8.2		

[a] Both compounds were administered 1 min prior to induction of arterial injury. [b] The results are expressed as percentage inhibition of the mean control value ± standard error of the mean ($n = 5$).

arterial model **42e** was appreciably more potent than MD-805 (Table 8), in both effect on platelet and fibrinogen accumulation, and again the difference was accentuated when using subcutaneous administration (Table 9). One point of interest with these data is that while the extension of APTT is mirrored by the inhibition of fibrinogen deposition, that is not the case with the platelet deposition. Here **42e** has a more profound effect on the platelet deposition than MD-805 for the same or lower extension of APTT. The differences observed with the different routes of administration may reflect a difference in bioavailability between the two compounds.

In summary, using MD-805 as a starting point, we have prepared a novel series of thrombin inhibitors with significantly improved in vitro potency over MD-805 but containing only one stereogenic center: that of the naturally occurring arginine. In animal models of both venous and arterial thrombosis, one of the compounds, **42e**, produced a dose-dependent inhibition of thrombus formation and demonstrated an improvement over MD-805 in the arterial situation.

Chemistry

P3 Sulfonyl Chlorides. *m*-Cumylphenylsulfonyl chloride ($mCPSO_2Cl$) was prepared from *o*-sulfanilic acid and 2-phenylpropene (Scheme 1) by an auto-acid-catalyzed alkylation, similar to that used for phenols.[28] The directing amino group was then removed from **1** by diazotization and reduction, followed by conversion of the sulfonic acid group via the sodium salt to the sulfonyl chloride **3**.

Racemic 3-monomethyltetrahydoquinolyl compounds (e.g. **39a**−**d**) were prepared according to literature procedure via 3-methylquinoline-8-sulfonyl chloride.[6a]

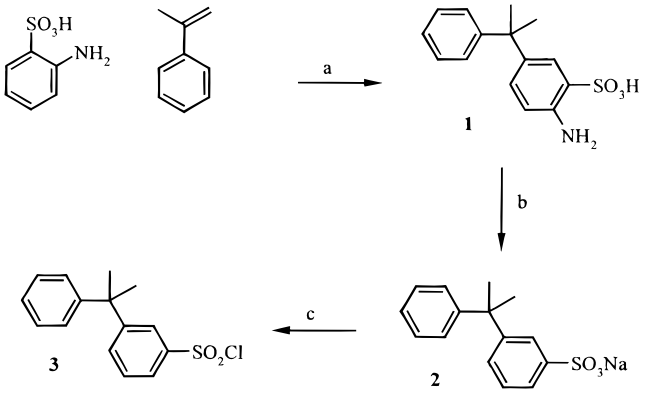
Mainly for reference purposes we wished to have access to a chiral MTHQS P3 and decided to synthesize stereospecifically 3(*R*)-methyl-6-bromotetrahydroquinolinesulfonyl chloride **8** as a reagent for inhibitor synthesis. From a retrosynthetic analysis of this chiral

Table 9. Effect of Subcutaneous Administration of **42e** and MD-805 in the Rat Model of Arterial Thrombosis

compd & dose (mg/kg)[a]	percentage inhibition[b]		APTT (multiple of control)[c]	
	platelets	fibrinogen	start	end
42e				
30.0	69.8 ± 4.1	49.9 ± 7.6	2.2 ± 0.2	2.5 ± 0.4
10.0	68.3 ± 3.1	45.5 ± 5.9	1.8 ± 0.1	1.7 ± 0.2
3.0	61.2 ± 5.6	28.8 ± 8.2	1.5 ± 0.1	1.3 ± 0.1
2.0	38.5 ± 14.5	3.5 ± 13.5	1.5 ± 0.1	1.0 ± 0.1
1.0	19.1± 11.7	24.2 ± 1.7	1.1 ± 0.1	1.0 ± 0.1
MD-805				
30.0	45.8 ± 5.8	57.4 ± 6.7	2.4 ± 0.2	2.4 ± 0.2
10.0	27.6 ± 11.8	37.0 ± 10.4	2.0 ± 0.1	1.7 ± 0.2
3.0	6.3 ± 21.0	12.3 ± 17.5	1.4 ± 0.2	1.2 ± 0.1

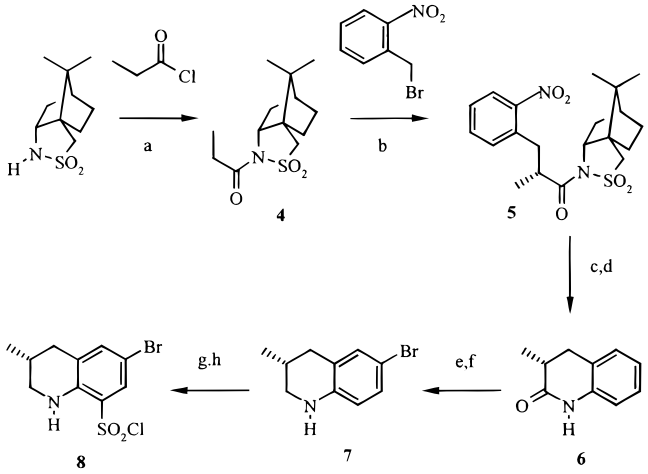
[a] Both compounds were administered 1 h prior to the induction of arterial injury. [b] Results are expressed as percentage inhibition of the mean control value ± standard error of the mean ($n = 5$). [c] APTT values are expressed as multiples of control and were measured just prior to clamping the artery (i.e. 1 h after dosing) and at the termination of the experiment (45 min later).

Scheme 1. Synthetic Route to *m*-Cumylphenylsulfonyl Chloride ($mCPSO_2Cl$)[a]



[a] Reaction conditions: (a) H_2O, reflux; (b) (i) HCl, H_2O, 65 °C, (ii) $NaNO_2$, 5 °C, (iii) H_3PO_2, 20−60 °C; (c) $SOCl_2$, DMF, 10−20 °C.

Scheme 2. Synthetic Route to 3(*R*)-Methyl-6-bromotetrahydroquinolinesulfonyl Chloride[a]



[a] Reaction conditions: (a) NaH, toluene, THF, 20 °C; (b) NaHMDS, THF, DMID, −78 °C; (c) H_2O_2, LiOH, THF, H_2O, 5−20 °C; (d) 10% Pd/C, cyclohexene, EtOH, reflux; (e) Br_2, $CHCl_3$, reflux; (f) $AlCl_3$, LAH, Et_2O, 20 °C; (g) Pyr-SO_3, Pyr, reflux; (h) $POCl_3$, Pyr, DMID, 20 °C.

intermediate it was envisaged that access could be gained by bromination[29] and reduction of an amide formed via the cyclization[30a,b] of an aminoaryl acid, which in turn would be obtained from reduction of a nitro acid, the final steps similar in effect to a route developed by Lassalle.[30c,d] The key step in our synthesis would be the diastereoselective alkylation of a suitable acid with a nitrobenzyl electrophile, and for this step we were attracted to the use of Oppolzer's sultam[31] because of the crystallinity of its derivatives. We are unaware of the prior use of this auxiliary with nitroaryl electrophiles.

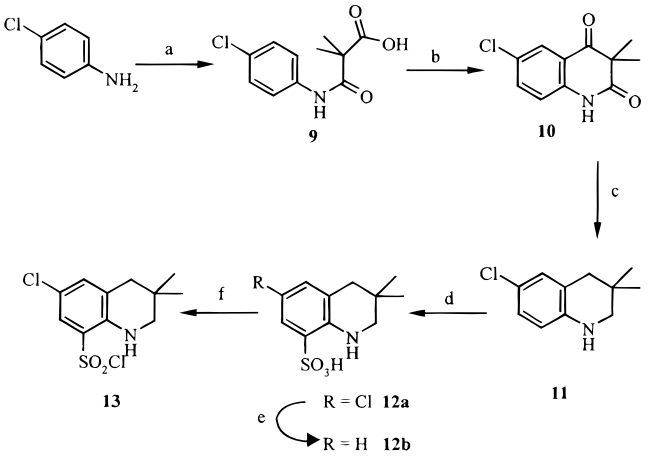
Thus, the acylsultam[32] **4** was alkylated with 2-nitrobenzylbromide (Scheme 2) to give, after chromatography, the alkylated product **5** in good yield. A single crystallization from methanol led to a compound with a diastereomeric ratio of >99%. The relative stereochemistry was proven by a single-crystal X-ray structure determination confirming that alkylation had occurred from the Si face of the chelated Z enolate.[31]

Mild lithium peroxide-mediated hydrolysis gave the crude acid which was directly reduced under phase-transfer conditions (10% Pd−C, cyclohexene) and cyclized to give the amide **6**. Bromination followed by LAH/$AlCl_3$[35] reduction gave the desired 3(*R*)-methyl-6-bromo-1,2,3,4-tetrahydroquinoline (**7**). Formation of the Mosher amide derivative[33] and NMR analysis showed **7** to be a single enantiomer.

The presence of dimethyl in the intermediate **12b** precludes the use of a simple quinoline sulfonation for this type of compound. A stepwise method was therefore developed for the construction of this ring system which incorporated the dimethyl group at an early stage (Scheme 3). Excess *p*-chloroaniline was acetylated with dimethylmalonic acid chloride (generated in situ) to give **9** in an acceptable yield, which was then cyclized cleanly to **10** with Eaton's[34] reagent. Simultaneous reduction of ketone and amide carbonyls with LAH in the presence of aluminum chloride[35] gave 6-chloro-3,3-dimethyltetrahydroquinoline (**11**).

An amino-directed ortho sulfonation of intermediates **7** and **11** with pyridine/SO_3 complex followed by treatment with $POCl_3$ led to the sulfonyl chlorides **8** and **13**. Initially this type of sulfonation had been carried out with sulfamic acid,[36] but pyridine/SO_3 complex was found to be superior. The 6-bromo and 6-chloro substituents, which had ensured the regioselectivity of sulfonation by blocking the position para to the amino group, could be removed either prior to the formation of the sulfonyl chloride (e.g. **12b**, Scheme 3) or later from the fully coupled inhibitor. It is possible that such a blocking group is not necessary, as sulfonate may be delivered regiospecifically to the ortho position by the amino group, but this was never examined.

Scheme 3. Synthetic Route to 3,3-Dimethyl-6-chlorotetrahydroquinolinesulfonyl Chloride (DMTHQSO$_2$Cl)[a]



[a] Reaction conditions: (a) dimethylmalonic acid, $SOCl_2$, THF, reflux, 20 °C; (b) P_2O_5, CH_3SO_3H, 70 °C; (c) $AlCl_3$, LAH, THF/Et_2O, 20 °C to reflux; (d) Pyr·SO_3, Pyr, reflux; (e) $NaHCO_3$, H_2O, 10% Pd/C, H_2; (f) (i) CH_3CN, ultrasound, (ii) Pyr, $POCl_3$, 20 °C.

P2 Piperidines. Hydroxyl-substituted piperidines **14**, obtained commercially or prepared by standard procedures,[37] were routinely protected as their *tert*-butyl ethers **15** or acetic esters **17** (Scheme 4) prior to coupling. N-Boc-protected 4-hydroxyethylpiperidine **18** (Scheme 5) was a key intermediate in the synthesis of a number of other 4-substituted piperidines; treatment with triphenylphosphine/CCl_4 or CBr_4 supplied the 4-chloroethyl- and 4-bromoethyl-substituted intermediates, respectively. Fluorination was achieved with DAST,[38] and oxidation to the intermediate aldehyde **23** facilitated difluorination.

Standard chemistry was employed to prepare additional 4-substituted piperidines from readily available pyridine precursors (Scheme 6): 4-formylpyridine was reacted with a stabilized phosphonium ylid to form the conjugated alkene ester **29**; concomitant reduction of the alkene and pyridine ring (H_2/Pt) led to piperidine **30**; reaction of the anion of 4-methylpyridine with a THP-protected 5-hydroxy-1-chloropentane **31** led via cyanide introduction to the chain-extended piperidinecarboxylic acid ethyl ester **34**. Further piperidine modifications were conducted on the fully assembled structures (Scheme 8).

Coupling Methods. A number of the simpler inhibitor structures were assembled by a sulfonyl choride coupling to L-arginine (Scheme 7), followed by conversion of the free carboxyl group to the acid chloride **35**−**37** and reaction with the appropriate piperidine. In the case of compounds containing methylquinolinesulfonyl (MQS) (prepared by literature procedure),[6a] catalytic reduction led to methyltetrahydroquinoline (MTHQS) P3-based structures **39a**−**d**.

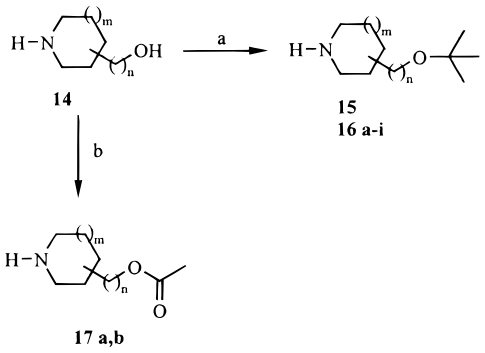
An alternative reversed procedure, used for the majority of inhibitors, was to couple the appropriate piperidine to α-N-boc-β-nitro-L-arginine via the mixed anhydride method. Removal of the Boc group followed by coupling of the liberated amine to P3 sulfonyl chloride led to a protected intermediate (**41**). Combined reduction of the quinoline ring (for MQS compounds) and reductive cleavage of the nitro protection furnished compounds **45a**−**j**. For certain test compounds further chemistry was carried out either prior to or following guanidine deprotection.

Experimental Section

Biology. Enzyme Assays: K_i Measurement. A stock solution of the experimental compound was prepared by dissolving the dry material to a concentration of 10 mg/mL in dimethyl sulfoxide (DMSO). In a preliminary range-finding experiment, a series of dilutions of the experimental compound stock solution (concentrations in the range 0.001−10 mg/mL) were diluted in a mixture of TRIS-HCl buffer (0.05 M, pH 8.4) and chromogenic substrate solution S-2238 (Chromogenix AB, Mölndal, Sweden; 12.5 μM) to give final concentrations of approximately 0.01−100 μM. Reactions were started by the addition of a solution of either human thrombin (Sigma T8885; 0.0625 U/mL final concentration) or bovine thrombin (Sigma T6634; 0.025 U/mL). The change in absorbance of the mixture was monitored for 30 s at 405 nm in a Perkin-Elmer Lambda16 spectrophotometer connected to a desktop computer running Perkin-Elmer UV Winlab software which calculated the slopes of the reaction curves. In this system, concentrations of inhibitor producing 20−80% inhibition of the reaction rate relative to the controls would be expected to fall in the range of approximately 1−4 times the inhibition constant (K_i). In all experiments, it was ensured that the DMSO concentration in the final reaction mixture was kept below 0.5%.

Where inhibition was apparent in the range-finding experiment, the basic procedure was repeated but using a series of different substrate concentrations in the range 3.125−100 μM with at least two different concentrations of experimental compound (within the range 1−4 times the expected K_i as determined in the range-finding experiment) and vehicle control tested for each substrate concentration. Reaction rates were again measured using the Perkin-Elmer Lambda16 spectrophotometer. The inhibition constant (K_i) for thrombin inhibition was derived from a Lineweaver−Burk plot of the

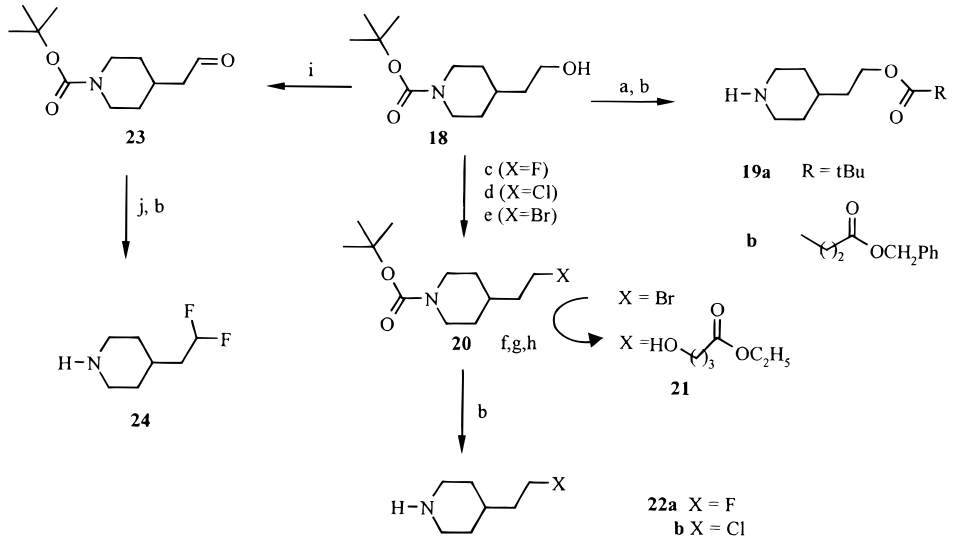
Scheme 4. Synthetic Routes to Protected Hydroxypiperidine Intermediates[a]



Intermediate	m	subst. position	n	config.
15	0	2	1	R
16 a	1	2	1	S
b	1	2	1	R
c	1	2	2	R,S
d	1	3	0	R,S
e	1	3	1	R
f	1	3	2	R
g	1	4	0	
h	1	4	2	
i	1	4	3	
17 a	1	4	1	
b	1	4	2	

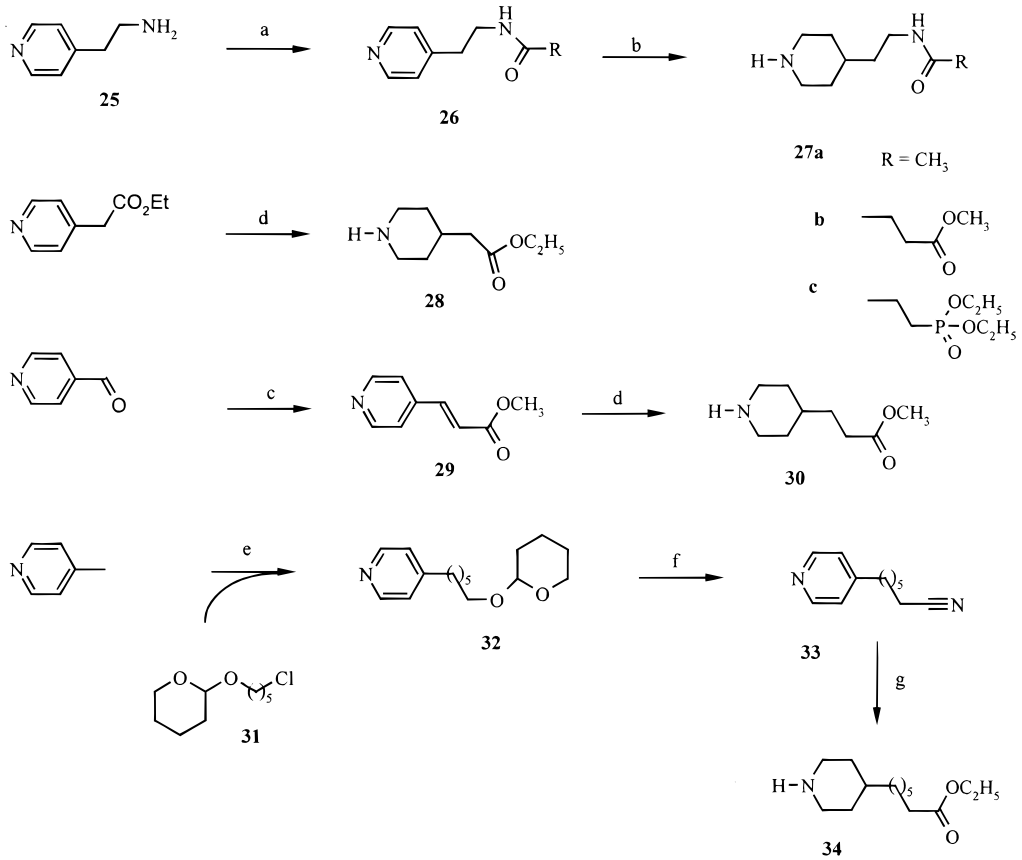
[a] Reaction conditions: (a) isobutylene, DCM, concd H_2SO_4, 5−20 °C; (b) AcOH, HCl(g), 5 °C−reflux.

Scheme 5. Synthetic Routes to Intermediate 4-Substituted Piperidines from Boc-Piperidine Ethanol[a]



[a] Reaction conditions: (a) RCOCl, DCM, Et_3N, pH > 9, −20 to 20 °C or RCOCl, pyridine, 5 °C; (b) HCl, AcOH, 20 °C; (c) $FCCl_3$, DAST, −78 to 20 °C; (d) DCM, CCl_4, PPh_3, 20 °C; (e) DCM, CBr_4, Ph_3P, 20 °C; (f) 1,4-butanediol, Na, xylene, 125 °C; (g) PDC, DMF, 20 °C; (h) EtOH, HCl; (i) PCC, DCM, 20 °C; (j) DCM, DAST.

Scheme 6. 4-Substituted Piperidine Intermediates[a]



[a] Reaction conditions: (a) RCOCl, DCM, TEA, pH > 9, −20 to 20 °C; (b) H_2, PtO_2, EtOH/4 M HCl (1 equiv), 20 °C; (c) $CH_3OOC{=}PPH_3$, DCM, 20 °C; (d) H_2, PtO_2, AcOH(gl), 20 °C; (e) ^{n}BuLi, THF, −78 °C; (f) (i) HCl, EtOH, 20 °C, (ii) $SOCl_2$, (iii) NaCN, DMSO; (g) (i) HCl, EtOH, (ii) H_2, 10% Pd/C, EtOH, 4 M HCl (1 equiv).

reciprocal of reaction rate versus the reciprocal of substrate concentration using the following formula:

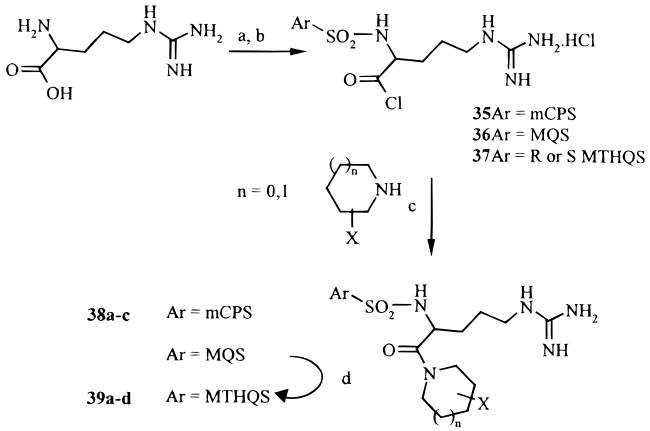
$$K_i = \frac{[i]}{\frac{K_p}{K_m} - 1}$$

where [i] = final concentration of inhibitor in the reaction cuvette, K_m = negative reciprocal of the intercept on the abscissa for the control curve, and K_p = apparent K_m in the presence of inhibitor at concentration *i* (negative reciprocal of the intercept on the abscissa for the inhibited curve).

Other enzyme assays were performed in a similar manner using the appropriate substrate: trypsin (S-2222), plasmin (S-2251), chymotrypsin (S-2586).

IC_{200} (APTT) Measurement. Freshly collected blood was immediately anticoagulated by mixing with one-tenth volume

Scheme 7. Synthesis Directly from Arginine: Coupling Method 1[a]



[a] Reaction conditions: (a) K_2CO_3, H_2O/dioxane (1:1), then $ArSO_2Cl$, 5−20 °C; (b) $SOCl_2$, 20 °C; (c) amine **3**−**5**/TEA, 1−5 °C, pH > 9; (d) H_2 (1 bar), 10% Pd/C, MeOH/AcOH/H_2O (30:3:1), 20 °C.

of trisodium citrate solution (3.8% w/v in distilled water). The blood was centrifuged at 1300*g* for 20 min to obtain platelet-poor plasma.

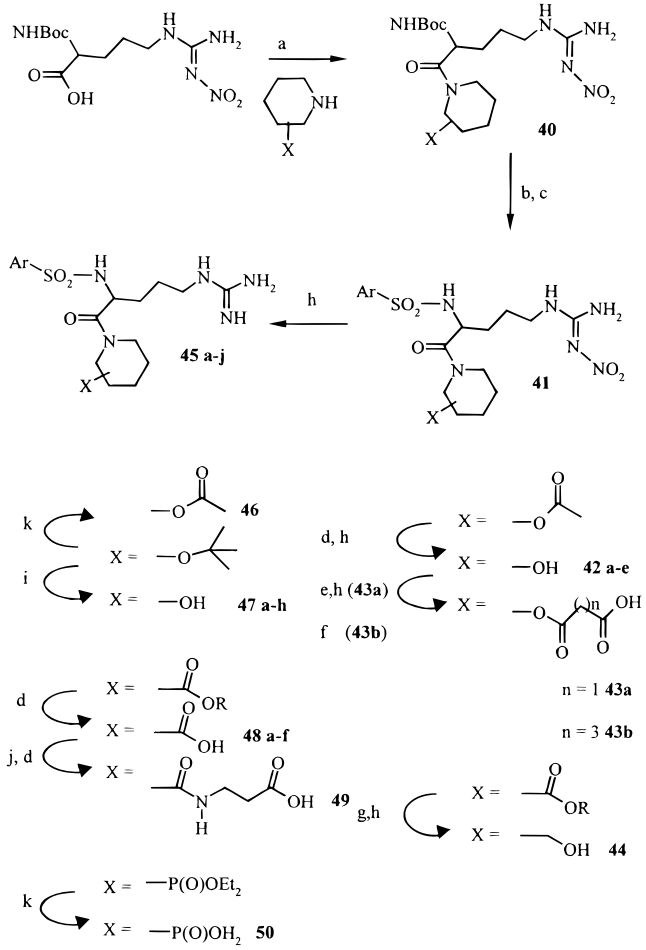
Aliquots of plasma were treated with solutions of experimental compound or vehicle alone to give a range of concentrations from 0 to approximately 150 μM. The APTTs of the treated plasma samples were determined using the standard method on the Instrumentation Laboratory ACL 300R coagulometer. The principle of the assay is that citrated plasma (50 Fl) was activated by incubating for 5 min at 37 °C with bovine cephalin reagent (Instrumentation Laboratory (UK) Ltd., APTT (ellagic acid) test kit; 50 FL), before initiating coagulation by the addition of calcium chloride solution (20 mM, 50 FL). The time taken for coagulation of the plasma to occur was measured automatically by the Instrumentation Laboratory ACL 300R coagulometer. The concentration of each compound required to double the APTT of the plasma was determined from a graph of concentration of experimental compound versus APTT.

Thrombosis Models. Rat venous thrombosis model: An acute model was used as previously described in the literature.[37] Systemic hypercoagulability was induced by the intravenous administration of a commercially available thromboplastin reagent (Thromboplastin C Dade; 20 μL/kg) prior to the initiation of stasis in the inferior vena cava by ligation. After 10 min the vessel was excised and weighed. The compounds were administered either 2 min (intravenous route) or 1 h (subcutaneous route) prior to the induction of stasis. Results were calculated from the difference in thrombus weight between placebo-treated and drug-treated animals expressed as percentage inhibition.

Rat arterial thrombosis model: An acute model of injury-induced thrombus formation in the rat dorsal aorta was used as described in the literature.[38] Rats were injected with 111-indium-labeled platelets (24 h) and 125-iodine-labeled fibrinogen (10 min) prior to inducing the injury. The injury to the dorsal aorta was caused by clamping the vessel with modified Spencer-Wells forceps for 1 min. Blood was then allowed to flow through the injured vessel for 45 min before the vessel was excised, washed in a 5% citrate solution, and counted in a γ-counter. Results were then expressed as mg blood equivalents by calculation from the counts in the circulating blood. Compounds were administered before clamping (iv 1 min before or sc 1 h before). The difference in radiolabel accumulation between drug- and placebo-treated animals was calculated and expressed as percentage inhibition.

Following subcutaneous administration of the compound, blood samples were removed via a carotid cannula into one-tenth volume trisodium citrate (3.8% w/v), just prior to clamping the dorsal aorta and at the termination of the experiment, to allow the determination of the plasma APTT as previously described.

Scheme 8. Synthesis from Boc-Protected Nitroarginine: Coupling Method 2[a]



[a] Reaction conditions: (a) DMF, NMM, ^{i}BuOCOCl, −15−20 °C, then amine selected from x to y; (b) (i) satd HCl in AcOH, 20 °C or (ii) 1 equiv HCl in DCM/AcOH (2:1), 0 °C; (c) DMF/DIPEA, pH > 9, $ArSO_2Cl$ (3-methylquinoline-8-sulfonyl chloride or **8** or **13**), 5−20 °C; (d) 1 M NaOH/MeOH (1:2); (e) acid chloride benzyl ester, Pyr, 20 °C; (f) glutaric anhydride, Pyr, −20 to 20 °C; (g) $LiBH_4$ in THF (2 M), reflux, then K_2CO_3, H_2O, 20 °C; (h) H_2 (1 bar), 10% Pd/C, MeOH/AcOH/H_2O (15:0.1:1); (i) TFA, HBr(g), 20 °C [or (b)(i)]; (j) H-AlaOEt, DCC, HOBt, DMF, TEA, 20 °C; (k) satd HBr/AcOH, 20 °C.

Chemistry. Reagent grade solvents were used without further purification unless specifically mentioned. Evaporation means removal of solvent by use of a Buchi rotary evaporator at a temperature of 40−50 °C in vacuo unless otherwise stated. Normal-phase silica gel used for flash purification was Kieselguhr-60 (230−400 mesh). TLC plates coated with silica gel 60 F_{254} (Merck) were used; detection was by UV (254), by iodine vapor, or by treatment with chlorine vapor followed by starch KI solution. HPLC analysis was carried out on a reverse-phase nucleosil C_{18} column unless otherwise stated. Proton and carbon NMR spectra were recorded on Bruker AMX 400-MHz and JEOL 90-MHz instruments; chemical shifts are quoted in ppm relative to tetramethysilane. Mass spectrometry was performed using a VG Trio 3000. Elemental analyses were carried out by Butterworth Laboratories.

2-Amino-3-(1-methyl-1-phenylethyl)benzenesulfonic Acid (1). (a) (Letters correspond with lettering from appropriate reaction scheme throughout the Experimental Section.) A mixture of 2-aminobenzenesulfonic acid (50 g, 0.29 mol) and 2-phenylpropene (34.3 g, 0.29 mol) in water was stirred and heated to reflux for 27 h. The reaction mixture was filtered hot, washing the collected product with portions of boiling

water (2 × 200 mL), ethanol (3 × 50 mL), and diethyl ether (2 × 100 mL) to yield **1** (52.4 g, 62%): mp = 274 °C; ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 6H), 7.2−7.5 (m, 7H), 7.7 (d, J = 2 Hz, 1H).

Sodium 3-(1-Methyl-1-phenylethyl)benzenesulfonate (2). (b) 2-Amino-3-(1-methyl-1-phenylethyl)benzene sulfonic acid (60 g, 0.206 mol) was suspended in water (412 mL), concd HCl (41 mL) added, and the mixture heated to 65 °C with stirring for 20 min. The mixture was cooled to room temperature, a further portion of concd HCl (41.4 mL) was added followed by the dropwise addition of sodium nitrite (14.3 g solution in water 41 mL), maintaining the temperature at 20 °C, and stirring continued for 30 min. Hypophosphorous acid (137 mL of 50 wt/wt) was added dropwise over 30 min and stirred at 20 °C for 1 h. The reaction mixture was gently warmed to 40 °C for 30 min, and the temperature then slowly increased to 60 °C (vigorous evolution of gas at 50−55 °C). The mixture stirred at this temperature for a further 30 min. After cooling to 20 °C the reaction mixture was filtered, evaporated to approximately 450 mL, and basified (pH 10−11) by the portionwise addition sodium hydroxide pellets. Water (300 mL) was added, the mixture warmed until dissolution was complete, and sodium chloride (80 g) added to aid crystallization. The product was filtered, washed with satd sodium chloride (50 mL), recrystallized from hot 15% sodium chloride solution (110 mL), filtered, washed with ice−water, and dried in a desiccator to yield **2** (35.5 g, 58%): ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 6H), 7.2−7.4 (m, 7H), 7.55 (d, J = 8 Hz,-1H), 7.63 (s, 1H).

3-(1-Methyl-1-phenylethyl)benzenesulfonyl Chloride (3). (c) Sodium 3-(1-methyl-1-phenylethyl)benzenesulfonate (35.5 g, 0.119 mol) was dissolved in DMF (60 mL) and cooled to 10 °C and thionyl chloride (44.6 mL) added dropwise maintaining the temperature below 20 °C until addition was complete. It was then stirred at 20 °C for 1 h. The reaction mixture was added to ice water (365 mL) and extracted with diethyl ether (3 × 275 mL). The combined ether extract was washed with water (275 mL), dried ($MgSO_4$), filtered, and evaporated. The resultant solid was redissolved in hexane (750 mL), treated with decolorizing charcoal, filtered, and evaporated to yield **3** as a pale yellow solid (29.1 g, 98.7 mmol, 83%): mp = 60 °C; TLC R_f 0.62 (EtOAc/hexane, 1:3); ^{1}H NMR ($CDCl_3$) δ 1.75 (s, 6H), 7.2 (m, 3H), 7.3 (m, 2H), 7.5 (m, 2H), 7.88 (d, J = 8 Hz, 1H), 8 (d, J = 1 Hz, 1H).

***N*-Propionyl-2(*S*)-bornane-2,10-sultam (4). (a)** A suspension of sodium hydride (2.01 g) in toluene (50 mL) was treated at 20 °C with a solution of 2(*S*)-bornane-10,2-sultam (9.85 g) in a mixture of THF (25 mL) and toluene (50 mL) added over 30 min. The mixture was stirred for 1 h during which a thick mass formed. Propionyl chloride (4.4 mL) in THF (20 mL) was added over 20 min and the mixture stirred for 16 h. Saturated aqueous ammonium chloride (50 mL) was added and the mixture poured into ether (50 mL); the separated aqueous layer was washed with ether (2 × 25 mL). The combined organic extracts were washed with portions (25 mL) of water and brine and dried ($MgSO_4$). Evaporation of the solvent gave a white solid (14.9 g) which was recrystallized from methanol to afford **4** (9.1 g, 73%): mp = 149.9−150.7 °C; ^{1}H NMR (500 MHz) (acetone-d_6) δ 1.00 (s, 3H), 1.08 (t, J = 7.3 Hz, 3H, CH_3−CH_2−C═O), 1.13 (s, 3H), 1.36 (m, 1H), 1.52 (m, 1H), 1.81 (br.t, J = 4 Hz, 1H), 1.87−2.05 (m, 4H), 2.64 (q, J = 7.35 Hz, 2H, CH_3-CH_2−C═O), 3.53 (d, J = 14.1 Hz, 1H), 3.68 (d, J = 14.1 Hz, 1H), 3.84−3.86 (dd, J = 5 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 8.81, 20.1, 21.3, 21.6, 27.0, 33.2, 39.3, 45.7, 48.4, 49.3, 53.3, 65.8, 172.7 (CONH).

***N*-[2(*R*)-Methyl-3-(2-nitrophenyl)propionyl]-2(*S*)-bornane-2,10-sultam (5). (b)** A cooled solution of *N*-propionyl-2(*S*)-bornane-2,10-sultam (10.26 g) in THF (120 mL) at −78 °C (all under nitrogen) was treated with NaHMDS (M solution) over 5 min dropwise. The mixture was stirred for 1 h at −78 °C, 2-nitrobenzyl bromide (12.5 g) in DMID (13.6 mL) added dropwise over 7−8 min, and the mixture allowed to warm to 5 °C over 3.5 h and then stirred at 20 °C for 16 h. Water (40 mL) was added and the mixture poured into ether (50 mL) and water (25 mL). The aqueous phase was extracted with ether (25 mL); the combined organic extracts werewashed with portions (2 × 25 mL) of water and brine and dried ($MgSO_4$). Evaporation of the solvent gave a viscous orange oil which was purified by flash chromatography (ether:hexane, 1:1 by vol., then ether) to afford a pale yellow solid (14.0 g, 91%) which crystallized from methanol to give two crops of **5** as pale yellow crystals, the main crop (8.12 g, 53%): mp = 126.5−127.5 °C; ^{1}H NMR (500 MHz) (acetone-d_6) δ 0.33 (s, 3H), 0.73 (s, 3H), 1.05 (d, J = 6.7 Hz, 3H), 1.08−1.15 (m, 1H), 1.25−1.33 (m, 1H), 1.45−1.52 (m, 2H), 1.58−1.78 (m, 3H), 2.93 (dd, J = 10.2 & 13.1 Hz, 1H), 3.19 (dd, J = 13.1 & 4.55 Hz, 1H), 3.30 (d, J = 14.1 Hz, 1H), 3.38 (d, J = 14.1 Hz, 1H), 3.35−3.47 (m, 1H), 3.60 (m, 1H), 7.2 (d, 1H), 7.3 (m, 1H), 7.45 (m, 1H), 7.85 (d, 1H); ^{13}C NMR (acetone-d_6) δ 17.69, 19.60, 20.74, 26.54, 32.76, 38.60, 38.79, 40.81, 45.31, 47.77, 48.55, 52.87, 65.37, 125.52, 128.70, 133.51, 133.86, 134.44, 149.80, 174.95.

3(*R*)-Methyl-3,4-dihydroquinolin-2-one (6). (c) *N*-[2(*R*)-Methyl-3-(2-nitrophenyl)propionyl]-2(*S*)-bornane-2,10-sultam (7.9 g) was dissolved in THF/water (4:1 by vol., 150 mL) at ice temperature (0−5 °C) and treated with hydrogen peroxide (30% w/v, 18.5 mL) followed by lithium hydroxide hydrate (3.64 g). The mixture was stirred at 0−5 °C for 1.3 h and then at 20 °C for 3.5 h. Aqueous hydrochloric acid (M, 150 mL) was added and the mixture poured into dichloromethane (100 mL). The aqueous phase was extracted with dichloromethane (2 × 50 mL), and the combined organic phases were extracted with 10% aqueous sodium bicarbonate (2 × 25 mL). The combined aqueous extracts were acidified to pH 1−2 with aqueous hydrochloric acid (M) and extracted with ether (4 × 25 mL), the combined extracts were dried ($MgSO_4$), and the solvent was evaporated to give 2(*R*)-methyl-3-(2-nitrophenyl)propionic acid as a pale yellow oil (3.9, 96%): ^{1}H NMR (90 MHz) (acetone-d_6) δ 1.21 (d, J = 6.8 Hz, 3H), 2.65−3.5 (m, 3H), 7.3−7.8 (m, 3H), 7.8−8.0 (m, 1H), which was used directly in the next step.

(d) A solution of 2(*R*)-methyl-3-(2-nitrophenyl)propionic acid (3.9 g) in ethanol (100 mL) was treated with cyclohexene (9.75 g) and 10% palladium on charcoal (3.8 g) and heated at reflux for 55 min. The mixture was cooled to 20 °C and filtered through a Celite pad to remove the catalyst and the filtrate evaporated to obtain a solid which was purified by flash chromatography (using ethyl acetate:dichloromethane, 1:19 by vol.) to give **6** as an off-white solid (2.01 g, 67%) which is sufficiently pure for use in the next step. Recrystallization from ether gave colorless crystals: mp = 129.9−131.8 °C; ^{1}H NMR (400 MHz) (acetone-d_6) δ 1.20 (d, J = 6.88 Hz, 3H), 2.49−2.58 (m, 1H), 2.69 (dd, J = 15.5 & 11.6 Hz, 1H), 2.98 (dd, J = 15.5 & 5.86 Hz, 1H), 6.91−6.95 (m, 2H), 7.13−7.18 (m, 2H).

6-Bromo-3(*R*)-methyl-1,2,3,4-tetrahydroquinoline (7). (e) 3(*R*)-Methyl-3,4-dihydroquinolin-2-one (1.98 g) was dissolved in chloroform (33 mL) and bromine (0.5 mL) added. The mixture was heated at reflux for 1 h and cooled to 20 °C; the solvent was evaporated to give a yellow oil which was purified by flash chromatography (ether/hexane, 65:35) to afford 6-bromo-3(*R*)-methyl-3,4-dihydroquinolin-2-one (2.33 g, 79%): mp = 147.0−148.3 °C; ^{1}H NMR (400 MHz) (acetone-d_6) δ 1.19 (d, J = 6.9 Hz, 3H), 2.5−2.61 (m, 1H), 2.72 (dd, J = 15.7 & 11.6 Hz), 3.02 (dd, J = 15.7 & 5.9 Hz), 6.88 (d, J = 8 Hz, 1H), 7.32 (dd, J = 8 & 2 Hz, 1H), 7.37 (d, J = 2 Hz, 1H).

(f) Aluminum chloride (1.198 g) was added over 5−10 min to dry ether (10 mL) at −10 °C under nitrogen. Lithium aluminum hydride (M in THF, 8.35 mL) was added to this solution and the resultant mixture stirred for 10 min at 20 °C. 6-Bromo-3(*R*)-methyl-3,4-dihydroquinolin-2-one (457 mg) in ether (30 mL) was then added over 10 min and the mixture stirred at 20 °C for 2 h. The mixture was cooled to −5 °C and water (5 mL) followed by aqueous sodium hydroxide (M, 10 mL). The mixture was extracted with ether (4 × 35 mL), the combined organic extracts were dried ($MgSO_4$), and the solvent was evaporated to give a colorless oil which solidified on standing. Flash chromatography (ether/hexane, 1:4) afforded **7** (0.39 g, 97%): ^{1}H NMR (400 MHz) (Acetone-d_6) δ 1.0 (d, J = 6.6 Hz, 3H), 1.88−2.0 (m, 1H), 2.32−2.39 (dd, J = 16 & 10

Hz, 1H), 2.69−2.75 (ddd, J = 16.0, 4.76 & 2.05 Hz, 1H), 2.80−2.87 (m, 1H), 3.23−3.29 (m, 1H), 5.2 (br.s, 1H), 6.42 (d, J = 8.8 Hz, 1H), 6.95−6.97 (m, 2H); $[\alpha]^{20} = -39.5°$ (c = 0.9, EtOH).

6-Bromo-3(*R*)-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl Chloride (8). (g) Pyridine/sulfur trioxide complex (593 mg) was added to a solution of 6-bromo-3(*R*)-methyl-1,2,3,4-tetrahydroquinoline (200 mg) in pyridine (8 mL) at 20 °C under nitrogen and the mixture heated at reflux for 23 h. After cooling to 20 °C, water (20 mL) was added and the mixture extracted with ether (2 × 10 mL). The combined extracts were washed with water (10 mL) and the combined aqueous layers evaporated to obtain a brown oil which was treated with aqueous hydrochloric acid (M, 20 mL). The off-white precipitate was collected by filtration and dried in vacuo overnight (NaOH pellets) to give as an off-white solid 6-bromo-3(*R*)-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic acid (177 mg, 65%) which was sufficiently pure for use in the next step: ^{1}H NMR (90 MHz) (DMSO-d_6) δ 0.97 (d, J = 6.3 Hz, 3H), 1.65−2.1 (m, 1H), 2.15−2.6 (m, 1H), 2.6−3.0 (m, 2H), 3.2−3.5 (m, 1H), 7.05 (d, J = 2.4 Hz, 1H), 7.40 (d, J = 2.4 Hz, 1H).

(h) 6-Bromo-3(*R*)-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic acid (177 mg) was suspended in DMID (5 mL) under an atmosphere of nitrogen at 20 °C and treated with pyridine (132 μL) followed by phosphoryl chloride (200 μL). The mixture was stirred at 20 °C for 3 h and then poured into ethyl acetate (20 mL) and extracted with water (2 × 10 mL). The combined aqueous layers were back=extracted with ethyl acetate (10 mL) and the combined organic extracts washed with brine (10 mL), dried ($MgSO_4$) and evaporated to give **8** as a yellow oil which contains a little DMID. The product was used immediately for the preparation of **42d**.

***N*-(4-Chlorophenyl)-2,2-dimethylmalonamic Acid (9). (a)** Dimethylmalonic acid (259 g) was dissolved in THF (500 mL), thionyl chloride (172 mL) added, and the mixture heated at reflux for 2 h and then cooled in ice. To this solution was added with stirring during 75 min at <10 °C a solution of 4-chloroaniline (500 g) in ether (500 mL). Stirring was continued for a further 30 min, ether (500 mL) added, and the mixture washed with aqueous hydrochloric acid (M, 3 × 1l) and extracted with aqueous sodium hydroxide (M, 3 × 1 L). Upon acidifying the combined basic extracts with concentrated aqueous hydrochloric acid an oil separated which, on keeping the mixture overnight at room temperature, partially crystallized. The crystals were collected by filtration and washed with water. Addition of more water to the stirred filtrate resulted in solution of the oil and the production of more crystals of **9** to yield in total 155 g (33% based on dimethylmalonic acid): mp = 169−170 °C; ^{1}H NMR (90 MHz) (CD_3OD) δ 1.53 (s, 6H), 5.18 (br.s, 1H, NH), 7.25 (m, J_{ortho}= 9.1 Hz, 2H), 7.54 (m, J_{ortho}= 9.1 Hz, 2H); ^{13}C NMR (90 MHz) (CD_3OD) δ 23.78, 51.68, 123.36, 129.42, 130.23, 137.98, 173.36, 177.04.

6-Chloro-3,3-dimethylquinoline-2,4-dione (10). (b) *N*-(4-Chlorophenyl)-2,2-dimethylmalonamic acid (150 g) was added in one charge to a stirred solution of phosphorus pentoxide (50 g) in methanesulfonic acid (1 L) at 70 °C under nitrogen. The mixture was stirred at 70 °C for 16 h, cooled to room temperature, and poured into ice−water (7.5 L) and the resulting suspension stirred for 1 h to afford material which was recovered by filtration and washing with cold water. The product was resuspended in cold water (4 L), stirred for 45 min, collected by filtration, washed, and dried in vacuo at 50 °C (P_2O_5) to yield **10** (133.3 g, 96%): mp = 227−228 °C; ^{1}H NMR (90 MHz) (DMSO-d_6) δ 1.33 (s, 6H), 4.25 (br.s, 1H, NH), 7.14 (dd, J_{ortho}= 7.2 Hz, 1H), 7.62 (dd, J_{ortho}= 7.2 Hz, 1H), 7.67 (s, 1H).

6-Chloro-3,3-dimethyl-1,2,3,4-tetrahydroquinoline (11). (c) Aluminum trichloride (86 g) was dissolved in dry ether (324 mL) and lithium aluminum hydride (M in THF, 800 mL) was added cautiously under vigorous stirring at room temperature. After stirring for a further 20 min, 6-chloro-3,3-dimethyl-1*H*-quinoline-2,4-dione (71.5 g) in dry THF (690 mL) was added at a rate to maintain a gentle reflux. After stirring for a further hour at room temperature, the mixture was cooled to −10 °C; water (2 L) and then aqueous sodium hydroxide (M, 1 L) were added cautiously to decompose the reagents and the ethereal layer was collected by decantation. The residue and aqueous supernatant were gently stirred with portions of ether (500 mL) which were collected by decantation until the extracts were colorless. The combined extracts were dried ($MgSO_4$), evaporated to dryness, and dried in vacuo (NaOH pellets) to give a solid which was crystallized from aqueous methanol to yield in two crops **11** (53 g, 84.7%): mp = 64−65 °C; ^{13}C NMR (90 MHz) ($CDCl_3$) δ 27.63, 29.04, 42.26, 54.39, 115.88, 122.27, 123.36, 127.74, 130.67, 143.24.

6-Chloro-3,3-dimethyl-1,2,3,4-tetrahydroquinoline-8-sulfonic Acid (12a). (d) Pyridine/sulfur trioxide complex (161 g) was added to a solution of 6-chloro-3,3-dimethyl-1,2,3,4-tetrahydroquinoline (45.7 g) in pyridine (1.72 L) and the mixture heated at reflux for 23 h. After cooling, the reaction mixture was poured into water (2 L) and the mixture extracted with ether (3 × 1 L). The combined ethereal extracts were back-extracted with water (3 × 500 mL) and the combined aqueous fractions evaporated to dryness. Addition of aqueous hydrochloric acid (M, 4.16l) to the brown oil gave a buff solid. After stirring for a further 15 min, the solid was collected by filtration, washed cautiously with a small amount of ice−water, and dried in vacuo (P_2O_5) to give **12a** (53.4 g, 83%): mp = 244−245 °C; single peak by RP HPLC 7 min (Nucleosil 10C_{18} CH_3CN/H_2O/TFA, 300:700:1); ^{1}H NMR (90 MHz) (DMSO-d_6) δ 0.93 (s, 6H), 2.47 (s, 2H), 2.99 (s, 2H), 6.98 (d, J = 4.5 Hz, 1H), 7.35 (d, J = 4.5 Hz, 1H); ^{13}C NMR (90 MHz) (DMSO-d_6) δ 25.73, 26.60, 40.31, 52.01, 120.10, 124.66, 124.98, 129.96, 132.02, 135.87.

3,3-Dimethyl-1,2,3,4-tetrahydroquinoline-8-sulfonic Acid (12b). (e) 6-Chloro-3,3-dimethyl-1,2,3,4-tetrahydroquinoline-8-sulfonic acid (7.46 g) was added to an aqueous sodium hydrogen carbonate solution (24.4 g in 630 mL water) and hydrogenated at a slight positive pressure for 3 days. The reaction mixture was filtered through Celite, washing with water, and evaporated to dryness. The residue was washed with 2-propanol and evaporated to dryness (×3), dissolved in ethanol, filtered, and evaporated (×2), with final evaporation from ether (×3) and drying in vacuo to give **12b** (4.6 g, 71%): single peak by RP HPLC 3.2 min (Zorbax C_8 CH_3CN/H_2O/TFA, 300:700:1); ^{1}H NMR (90 MHz) (D_2O) δ 0.95 (s, 6H), 2.62 (s, 2H), 2.81 (s, 2H), 6.6 (dd, J = 3 Hz, 1H), 7.1 (d, J = 3 Hz, 1H), 7.45 (d, J = 3 Hz); ^{13}C (D_2O) δ 27.95, 28.8, 43.5, 54.9, 117.5, 125.7, 127.8, 135.8.

6-Chloro-3,3-dimethyl-1,2,3,4-tetrahydroquinoline-8-sulfonyl Chloride (13). (f) 6-Chloro-3,3-dimethyl-1,2,3,4-tetrahydroquinoline-8-sulfonic acid (2.0 g) was suspended in acetonitrile (24 mL) and ultrasonicated for 10 min. Pyridine (1.2 mL) was added and the solution covered with a blanket of nitrogen. After 10 min, phosphoryl chloride (1.34 mL) was added dropwise and the mixture stirred for 16 h at 20 °C. Following evaporation of the solvents, the residue was partitioned between ethyl acetate and aqueous sodium bicarbonate. The organic phase was washed with portions (25 mL) of aqueous sodium bicarbonate and water (×2), dried ($MgSO_4$), and evaporated to give **13** which was dried and stored in vacuo (NaOH pellets), with no further purification prior to use.

2(*S*)-*tert*-Butoxymethylpiperidine (16a). Piperidin-2(*S*)-ylmethanol (6.45 g) in dichloromethane (140 mL) was cooled to 0−5 °C and concentrated sulfuric acid (5.5 g) added dropwise with vigorous stirring. Isobutylene was bubbled through the mixture for 1 h with the temperature maintained at 0−5 °C. Concentrated sulfuric acid (1.37 g) was added dropwise; the mixture was enclosed securely and allowed to warm to room temperature with vigorous stirring for 5 h. The reaction mixture was cooled to 0−5 °C and aqueous potassium carbonate (50% w/v, 85 mL) added slowly, followed by diethyl ether (85 mL). The organic layer was separated and the aqueous phase washed with diethyl ether (2 × 75 mL). The combined organic layers were washed with brine (2 × 50 mL), dried ($MgSO_4$), filtered, and evaporated to give an oil which was distilled. The fraction boiling at 66−7 °C at 3.5 mmHg was collected to give **16a**: $[a]_D^{26}$ + 20.5° (c = 2, ethanol).

Intermediates **15** and **16b**−**16i** were prepared by an analogous procedure to **16a** but using the respective piperidine alcohols.

Acetic Acid 2-Piperidin-4-ylethyl Ester (17b). Piperidin-4-ylethanol (50 g) was dissolved in acetic acid (1 L) and cooled in ice. Hydrogen chloride gas was passed through the solution for 2 h; the reaction mixture was sealed and kept at 20 °C for 16 h and finally heated at reflux for 30 min. After cooling, the solvent was removed by evaporation and the residue held in vacuo (NaOH pellets) to give an oil which was stirred with ether (1 L) to give crystals: mp 80−90 °C. Recrystallization from ethyl acetate afforded **17b** hydrochloride (67.23 g, 83.6%): mp 115−117 °C; TLC $R_f = 0.36$ ($CHCl_3$/MeOH, 10:1); ^{13}C NMR (dioxane-d_8) δ 21.2, 28.8, 30.8, 34.4, 44.8, 63.5 (CH_2O), 174.8 (C═O). Anal. ($C_9H_{18}NO_2Cl$) C, H, N, Cl.

Intermediate **17a** was prepared by an analogous procedure to **17b** from piperidin-4-ylmethanol.

2,2-Dimethylpropionic Acid 2-Piperidin-4-ylethyl Ester (19a). (a) 4-(2-Hydroxyethyl)piperidine-1-carboxylic acid *tert*-butyl ester (7.15 g) was dissolved in dry pyridine (100 mL) and 2,2-dimethylpropionyl chloride (9.64 g) added at 0 °C. The mixture was stirred for 16 h and the solvent removed by rotary evaporation. Portions (3 × 50 mL) of ethanol were evaporated from the residue which was then partitioned between ethyl acetate and water (100 mL of each) and separated, the aqueous layer was re-extracted with ethyl acetate, and the combined organics were washed with water, brine (×2), dried ($MgSO_4$), and evaporated to give 4-[2-(2,2-dimethylpropionyloxy)ethyl]piperidine-1-carboxylic acid *tert*-butyl ester as an oil (9.7 g, 99.8%): TLC $R_f = 0.51$ (CH_2Cl_2/EtOAc, 9.5:1).

(b) 4-[2-(2,2-Dimethylpropionyloxy)ethyl]piperidine-1-carboxylic acid *tert*-butyl ester (4.45 g) was dissolved in a solution of hydrogen chloride in acetic acid (60 mL of 1 M) and the solution stirred for 2 h at 20 °C. The solvents were removed by rotary evaporation at reduced pressure and the residue was triturated with dry ether and dried in vacuo give the hydrochloride salt of **19a** as a white solid (3.37 g, 96.5%): ^1H NMR (400 MHz) (D_2O) δ 1.18 (s, 9H, tBu), 1.46 (m, 2H), 1.68 (m, 2H), 1.75 (m, 1H), 1.98 (m, 2H), 2.98 (m, 2H), 3.42 (m, 2H), 4.17 (t, $J = 6.2$ Hz, 2H, CH_2O); ^{13}C NMR (acetone-d_6) δ 29.16 (tBu), 30.96, 33.14, 36.55, 41.43 (quarternary), 46.76, 65.56 (CH_2O).

Intermediate **19b** was prepared by an analogous procedure to **19a** but using 3-chlorocarbonylpropionic acid benzyl ester as the acylating agent.

4-(2-Bromoethyl)piperidine-1-carboxylic Acid *tert*-Butyl Ester (20). (X = Br). (e) 4-(2-Hydroxyethyl)piperidine-1-carboxylic acid *tert*-butyl ester (18.7 g) was dissolved in dry dichloromethane (50 mL) and carbon tetrabromide (40.7 g) added, followed by triphenylphosphine (21.4 g) in dry dichloromethane (50 mL) slowly over 2 h. The mixture was stirred at 20 °C for 2 h, hexane (500 mL) was added to the mixture which was washed with portions (2 × 100 mL) of saturated aqueous sodium bicarbonate, water, and brine and dried ($MgSO_4$), and the solvents were evaporated to give an oil which was purified by flash chromatography (diethyl ether/hexane, 1:9 followed by 3:7) to afford **20** as an oil which slowly crystallized on storage (15.5 g, 65%): mp 39−42 °C; TLC $R_f =$ 0.5 (diethyl ether/hexane, 3:7); ^1H NMR (90 MHz) (acetone-d_6) δ 0.9−1.35 (m, 2H), 1.42 (s, 9H), 1.5−1.9 (m, 4H), 2.7 (td, $J = 13.6$ Hz, $J = 3.4$ Hz, 2H), 3.52 (t, $J = 7$ Hz, 2H), 3.9−4.2 (m, 2H); ^{13}C NMR (acetone-d_6) δ 29.0, 32.2, 32.5, 35.6, 40.4, 44.8, 79.4 (CH_2Br) 155.1 (C═O).

4-(2-Piperidin-4-ylethoxy)butyric Acid Ethyl Ester (21). (f) 1,4-Butanediol (3.52 mL) and sodium (0.46 g) were heated at 125 °C in xylene (10 mL) to give a solution to which 4-(2-bromoethyl)piperidine-1-carboxylic acid *tert*-butyl ester (5.84 g) was added. The mixture was heated at 125 °C for 16 h, the solvent evaporated, and the residue partitioned between chloroform and water. Evaporation of the organic phase gave crude material which was purified by flash chromatography (ethyl acetate) to give 4-[2-(4-hydroxybutoxy)ethyl]piperidine-1-carboxylic acid *tert*-butyl ester (3.4 g, 56%): TLC $R_f = 0.58$ (EtOAc); ^{13}C NMR ($CDCl_3$) δ 26.1, 28.2, 29.4, 31.9, 32.7, 36.0, 43.7, 61.6, 67.9, 70.5, 78.8, 154.4.

(g) A solution of 4-[2-(4-hydroxybutoxy)ethyl]piperidine-1-carboxylic *tert*-butyl ester (1.44 g) was added in DMF (10 mL) to a solution of pyridinium dichromate (6.3 g) in DMF (20 mL) and stirred for 16 h at 20 °C. The mixture was then poured into water (400 mL) and extracted with ether (3 × 50 mL), the combined extracts were dried (Na_2SO_4), and the solvent was evaporated to give 4-[2-(3-carboxypropoxy)ethyl]piperidine-1-carboxylic acid *tert*-butyl ester (1.41 g, 93%): TLC R_f = 0.38 (EtOAc); ^1H NMR ($CDCl_3$) δ 1.03 (m, 2H), 1.38 (s, 9H), 1.43 (m, 3H), 1.60 (m, 2H), 1.82 (t, $J = 6.5$ Hz, 2H), 2.38 (t, J = 6.5 Hz, 2H), 2.61 (m, 2H), 3.40 (t, $J = 6.5$ Hz, 4H), 4.00 (br.m, 2H); ^{13}C NMR ($CDCl_3$) δ 25.2, 25.4, 28.8, 31.3, 31.4, 32.5, 33.3, 36.4, 36.5, 68.9, 70.1, 155.4, 174.0. $m/e = 315.9$ $(M + H)^+$.

(h) 4-[2-(3-Carboxypropoxy)ethyl]piperidine-1-carboxylic acid *tert*-butyl ester (1.41 g) was dissolved in ethanol (50 mL) and hydrogen chloride gas bubbled slowly through the solution for 3 h. The solvent was evaporated to give **21** as the hydrochloride salt (1.1 g, 88%): ^1H NMR ($CDCl_3$) δ 0.78 (m, 2H), 1.25 (t, $J =$ 7 Hz, 3H), 1.51−1.82 (br.m, 3H), 1.82−2.0 (m, 4H), 2.35 (t, J = 7 Hz 2H), 2.9 (br. s 2H),3.4−3.6 (br.m, 5H), 3.73 (q, $J = 7$ Hz, 1H), 4.14 (q,$J = 7$ Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.6, 25.4, 29.1, 31.4, 31.5, 35.5, 44.7, 60.7, 68, 70.2, 173.8; $m/e =$ 243.9 $(M + H)^+$.

4-(2-Fluoroethyl)piperidine (22a). (c) 4-(2-Hydroxyethyl)piperidine-1-carboxylic *tert*-butyl ester (5.15 g) was dissolved in fluorotrichloromethane (15 mL) and cooled to −78 °C in an atmosphere of dry nitrogen. A solution of DAST (3.55 mL) in fluorotrichloromethane (15 mL) was added and the mixture stirred with exclusion of moisture for 10 min at −78 °C before being allowed to warm to room temperature. After a further 30 min, the mixture was poured into ice−water (30 mL) and the organic phase separated, washed with brine (2 × 10 mL), and dried ($MgSO_4$). The solvent was removed by evaporation at reduced pressure to give a residue which was purified by flash chromatography (hexane/diethyl ether, 9:1). The appropriate fractions were combined and the solvent was removed to give 4-(2-fluoroethyl)piperidine-1-carboxylic acid *tert*-butyl ester (**20**, X = F) (2.2 g, 43%): TLC $R_f = 0.25$ (hexane/ether, 9:1); ^1H NMR (90 MHz) (acetone-d_6) δ 1.45 (s, 9H), 2.72 (ddd, $J = 12$ Hz, 12 Hz, 2 Hz, 4H), 3.9−4.3 (m, 4H), 4.58 (dt, $J_{HF} = 48$ Hz, $J_{HH} = 6.9$ Hz, 2H).

(b) 4-(2-Fluoroethyl)piperidine-1-carboxylic acid *tert*-butyl ester (2.2 g) was dissolved in saturated hydrogen chloride in acetic acid (12 mL) and the solution stirred for 2 h at 20 °C. The solvent was removed by evaporation at reduced pressure and portions of methanol (2 × 50 mL) were evaporated from the residue to give **22a** hydrochloride which was stored in vacuo (NaOH pellets) prior to use.

4-(2-Chloroethyl)piperidine (22b). (d) 4-(2-Hydroxyethyl)piperidine-1-carboxylic acid *tert*-butyl ester (29.5 g) was dissolved in dry dichloromethane (78.4 mL) and carbon tetrachloride (12.4 mL) added. Triphenylphosphine (33.8 g) was added in dry dichloromethane (78 mL) slowly over 1 h; the mixture stirred at 20 °C for 3 h. A further 0.5 equiv of carbon tetrachloride was added and the reaction mixture stirred at 20 °C for 16 h. Hexane was added to turbidity, the mixture was washed with portions (2 × 100 mL) of saturated aqueous sodium bicarbonate solution and brine and dried ($MgSO_4$), and the solvents were evaporated to give an oily white solid which was purified by flash chromatography (diethyl ether/hexane 3:7) to afford 4-(2-chloroethyl)piperidine-1-carboxylic acid *tert*-butyl ester (**20**, X = Cl) as a white solid (15.5 g, 48.5%): mp = 49.5−51 °C; TLC $R_f = 0.21$ (hexane/ether, 7:3).

(b) 4-(2-Chloroethyl)piperidine-1-carboxylic acid *tert*-butyl ester (**20**, X = Cl) (3.5 g) was dissolved in saturated hydrogen chloride in acetic acid (28 mL) and the solution stirred for 2 h at 20 °C. The solvents were removed by rotary evaporation at reduced pressure and the residue was triturated with dry ether to give **22b** hydrochloride as a white solid (2.9 g, 100%): TLC $R_f = 0.15$ ($CHCl_3$/MeOH/AcOH, 6:1:1).

4-(2-Oxoethyl)piperidine-1-carboxylic Acid *tert*-Butyl Ester (23). (i) Pyridinium chlorochromate (1.41 g) was suspended in dry dichloromethane (10 mL) and 4-(2-hydroxyethyl)piperidine-1-carboxylic acid *tert*-butyl ester (1.0 g) dissolved in dichloromethane (2 mL) added. The mixture was stirred at 20 °C for 6 h, ether (10 mL) added, and the mixture filtered through a pad of Celite. The filtrate was dried by rotary evaporation and the crude product purified by flash chromatography (ethyl acetate) to give **23** (0.85 g, 85%): TLC R_f = 0.58 (EtOAc); ^{1}H NMR (90 MHz) (acetone-d_6) δ 1.35 (s, 9H), 2.75 (m, 4H), 4.0 (m, 4H), 10.0 (1H, CHO); IR d 1728(−CH═O).

4-(2,2-Difluoroethyl)piperidine (24). (j) 4-(2-Oxoethyl)-piperidine-1-carboxylic acid *tert*-butyl ester (6.8 g) was dissolved in dry dichloromethane (2 mL) in an atmosphere of nitrogen. DAST (1 mL) was added and the reaction mixture stirred at 20 °C for 1 h and then poured into water (10 mL). The mixture was extracted with dichloromethane (2 × 10 mL), the combined extracts were dried ($MgSO_4$), and the solvent was removed by rotary evaporation to give crude product which was purified by flash chromatography (hexane/diethyl ether, 1:1) to give 4-(2,2-difluoroethyl)piperidine-1-carboxylic acid *tert*-butyl ester (4.22 g, 58%): TLC R_f = 0.42 (hexane/ether, 1:1); ^{1}H NMR (90 MHz) (acetone-d_6) δ 1.52 (s, 9H), 2.9 (m, 4H), 4.2 (m, 4H), 6.2 (tt, J_{HF} = 40 Hz, J_{HH} = 3 Hz, 1H).

(b) 4-(2,2-Difluoroethyl)piperidine-1-carboxylic acid *tert*-butyl ester was dissolved in acetic acid saturated with hydrogen chloride (12 mL) and the solution stirred for 2 h at 20 °C. The solvent was removed by evaporation at reduced pressure and portions of methanol (2 × 50 mL) were evaporated from the residue to give **24** as the hydrochloride salt.

***N*-(2-Pyridin-4-yl)ethyl)succinamic Acid Methyl Ester (26b) (R = $(CH_2)_2COOCH_3$). (a)** 3-Chlorocarbonylpropionic acid methyl ester (6.8 mL) was dissolved in dichloromethane (65 mL) at −20 °C with exclusion of moisture ($CaCl_2$ tube). 2-Pyridin-4-ylethylamine (5.04 g) and triethylamine (6.2 mL) were added in two equal portions and the mixture stirred at 20 °C for 16 h before filtration and evaporation of the filtrate to dryness. The resultant residue was purified by flash chromatography (ethyl acetate as eluent) to give **26b** (R = $(CH_2)_2COOCH_3$) as a light orange solid (7.65 g, 78.5%): ^{1}H NMR ($CDCl_3$) δ 2.39 (t J = 6.5 Hz, 2H), 2.58 (t, J = 6.5 Hz, 2H), 2.74 (t, J = 7 Hz, 2H), 3.44 (t, J = 7 Hz, 2H), 6.3 (br.s 1H), 7.06 (d, J = 6 Hz, 2H), 8.4 (d, J = 6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 29.6, 31.2, 35.4, 40.2, 52.2, 124.7, 148.8, 150.0, 172.0, 173.9; m/e = 236.9 $(M + H)^+$.

***N*-(2-Piperidin-4-ylethyl)succinamic Acid Methyl Ester (27b). (b)** *N*-(2-Pyridin-4-ylethyl)succinamic acid methyl ester (7.5 g) was dissolved in ethanol (100 mL) and aqueous hydrochloric acid (4 M, 1 equiv) added. The mixture was hydrogenated in the presence of Adam's catalyst (1.2 g) for 16 h at 20 °C and the catalyst removed by filtration through a pad of Celite. Evaporation of the filtrate gave **27b** hydrochloride as a colorless oil (8.8 g, 100%) which was stored in vacuo (NaOH pellets): ^{1}H NMR ($CDCl_3$) δ 1.5−1.72 (m, 5H), 1.93 (br.m, 2H), 2.51 (t, J = 7 Hz, 2H), 2.65 (t, J = 7 Hz, 2H), 2.93 (br.m, 2H), 3.27 (t, J = 7 Hz, 2H), 3.42 (br.m, 2H) 3.70 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 28.7, 29.7, 30.9, 31.7, 35.6, 36.9, 44.4, 52.2, 172.7, 174.0; m/e = 242.9 $(M + H)^+$.

27a was prepared by an analogous procedure to **27b**.

[2-(2-Pyridin-4-ylethylcarbamoyl)ethyl]phosphonic Acid Diethyl Ester (26c) (R = $(CH_2)_2P(O)(OEt)_2$). (a) Aminoethylpyridine (0.718 g) was dissolved in DMF (10 mL) and cooled to 0 °C and triethylamine (0.78 mL, 1 equiv) added, followed by (2-chlorocarboxyethyl)phosphonic acid diethyl ester (1.28 g) in DMF (2 mL). The reaction mixture, which was at pH 8−9 was stirred overnight, evaporated to dryness and the residue extracted with $CHCl_3$, evaporated, and purified by flash chromatography ($CHCl_3$/MeOH/HOAc, 6:0.5:0.5) to give after evaporation **26c** (R = $(CH_2)_2P(O)(OEt)_2$) (0.38 g, 21.5%): ^{13}C NMR ($CDCl_3$) δ 16.34, 16.6, 24.3, 28.8, 29.0, 35.1, 39.9, 62.1, 62.3, 124.6, 148.7, 149.6, 171.4, 172.0. This material was carried on to the next step without further purification.

[2-(2-Pyridin-4-ylethylcarbamoyl)ethyl]phosphonic Acid Diethyl Ester (27c). (b) [2-(2-Pyridin-4-ylethylcarbamoyl)ethyl]phosphonic acid diethyl ester (**26c)** (R = $(CH_2)_2P(O)(OEt)_2$) (0.35 g) was dissolved in methanol:acetic acid (9:1, by vol., 5 mL) and hydrogenated (1 bar) at 20 °C for 16 h in the presence of 10% palladium on charcoal (25 mg). The catalyst was removed by filtration and the filtrate dried by rotary evaporation to give **27c** acetate salt (0.35 g, 100%): ^{13}C NMR ($CDCl_3$) δ 15.7, 16.0, 17.4, 23.7, 28.0, 30.7, 34.7, 44.0, 62.0, 62.3, 171.8, 172.5; R_f = 0.22 (BuOH/AcOH/H_2O, 6:1:1). This material was stored in vacuo (NaOH pellets) prior to use.

Ethyl 4-Piperidinylacetate Hydrochloride (28). (d) By hydrogenation conditions analogous to those used for **27b** (part b) ethyl 4-pyridylacetate was converted to **28**: ^{1}H NMR (MeOH-d_4) δ 1.22 (t, J = 7 Hz, 3H), 1.45 (d, J = 13 Hz, 2H), 1.92 (d, J = 14 Hz, 2H), 2.00−2.13 (m, 1H), 2.32 (d, J = 7 Hz, 2H), 3.00 (t, J = 13 Hz, 2H), 3.35 (d, J = 13 Hz, 2H), 4.12 (d, J = 13 Hz, 2H); ^{13}C NMR (MeOH-d_4) δ 15.0, 30.2, 32.6, 41.6, 45.6, 62.1, 173.9; m/e = 171.8 $(M + H)^+$.

Methyl 3-(Pyrid-4-yl)prop-2-enoate (29). (c) To a stirred solution of carbomethoxymethylenetriphenylphosphorane (28.9 g) in CH_2Cl_2 (53 mL) at 0−5 °C under a nitrogen atmosphere was gradually added (exothermic) a solution of 4-pyridinecarboxaldehyde (11.6 g of freshly distilled) in CH_2Cl_2 (53 mL). The reaction mixture was refluxed for 3 h, cooled, and evaporated to dryness. The residue was extracted repeatedly with hot petrol (60−80), cooled, and filtered. The filtrate was reduced in volume and allowed to crystallize to yield two crops of **29** with identical melting points (72 °C) which were combined (10.2 g, 72.8%): TLC R_f = 0.15 (hexane/EtOAc, 1:1), 0.46 ($CHCl_3$/MeOH/AcOH, 6:1:1); ^{13}C NMR ($CDCl_3$) δ 52.3, 122.9, 142.3, 151.0, 166.7.

Methyl 3-(Piperidin-4-yl)propanoate (30). (d) Methyl 3-(pyridin-4-yl)prop-2-enoate (10.2 g) was stirred with platinum oxide (0.5 g) in glacial acetic acid (110 mL) under an atmosphere of hydrogen gas at 20 °C for 30 h. When the reaction was judged complete by TLC the catalyst was filtered off on a pad of Celite, the filtrate neutralized (aq $NaHCO_3$ soln) and evaporated to dryness. The residue was stirred with $CHCl_3$ (50 mL), insoluble material filtered off, and the filtrate evaporated to dryness, with final pumping over NaOH pellets to yield **30** (6.6 g, 61%): TLC R_f = 0.29 ($CHCl_3$/MeOH/AcOH, 6:1:1).

2-[6-(4-Pyridyl)hexyl-1-oxy]tetrahydropyran (32). (e) To a solution of 4-picoline (2.82 mL, 29 mmol) in THF (50 mL) at −78 °C was added dropwise *n*-butyllithium (15.6 mL of 2.5M, 39 mmol) to form an orange solution, which was stirred for a further 30 min at this temperature prior to the addition of 5-(tetrahydropyran-2-yloxy)pent-1-yl chloride (**31**) (5.4 g, 26 mmol). The reaction mixture was stirred at −78 °C for 1 h and at 20 °C for 18 h, quenched with satd NH_4Cl solution, and extracted with ethyl acetate (2 × 150 mL). The combined organics were washed with satd NaCl solution (2 × 50 mL), dried ($MgSO_4$), filtered, and evaporated to yield an orange oil (8.123 g). This was purified by flash chromatography (EtOAc/hexane, 2:3) to yield **32** (3.18 g, 46%): ^{1}H NMR ($CDCl_3$) δ 1.2−1.9 (m, 14H), 2.6 (t, J = 8 Hz, 2H), 3.3−3.54 (2m, 2H), 3.62−3.90 (2m, 2H) 4.55 (t, J = 5 Hz, 1H), 7.1 (d, J = 6 Hz, 2H), 8.5 (d, J = 6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 20.1, 25.9, 26.6, 29.4, 30.0, 30.6, 31.2, 35.5, 62.8, 67.9, 99.3, 124.3, 150.0, 152.0; m/e = 263.9 $(M + H)^+$.

7-(Pyridin-4-yl)heptane-1-nitrile (33). (f) (i) 2-[6-(4-Pyridyl)hexyl-1-oxy]tetrahydropyran (**32**) (3.15 g, 12 mmol) was dissolved in ethanol (20 mL), concd HCl (1.5 mL) added, and the reaction mixture stirred at 20 °C for 48 h. The solvent was evaporated; the orange residue was dissolved in water (20 mL), basified (pH 8) with satd $NaHCO_3$ solution, and extracted with dichloromethane (3 × 250 mL). The combined organics were dried ($MgSO_4$), filtered, and evaporated to yield 6-(4-pyridyl)hexan-1-ol (2.2 g, 85%) which was used without further purification: ^{1}H NMR ($CDCl_3$) δ 1.0−1.72 (2m, 8H), 2.30 (br.s, 1H), 2.58 (t, J = 7.5 Hz, 2H), 3.6 (m, 2H), 7.13 (br.s, 2H), 8.45 (br.s, 2H); ^{13}C NMR ($CDCl_3$) δ 26.0, 29.3, 30.6, 33.0, 35.5, 63.1, 124.4, 149.9, 152.2; m/e = 179.9 $(M + H)$.

(ii) Thionyl chloride (1.03 mL) was added to 6-pyridin-4-ylhexan-1-ol hydrochloride (2.42 g) and the mixture stirred at 20 °C for 30 min. An excess of saturated aqueous sodium bicarbonate solution and chloroform (20 mL) were added, and the mixture was shaken. The organic phase was separated, washed with saturated aqueous sodium bicarbonate, brine, and water, and dried ($MgSO_4$). The solvent was evaporated to give crude material which was then purified by flash chromatography (methanol/chloroform = 1:49) to give 4-(6-chlorohexyl)-pyridine as an oil (1.71 g, 64.1%): ^{1}H NMR ($CDCl_3$) δ 1.38 (m, 2H),1.48 (m, 2H), 1.65 (quintet J = 7.7 Hz, 2H), 1.75 (quintet, J = 7.5 Hz, 2H), 2.60 (t, J = 7.5 Hz, 2H), 3.52 (t, J = 7.5 Hz, 2H), 7.20 (d, J = 6 Hz, 2H), 8.50 (d, J = 6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 27.1, 28.8, 30.5, 32.8, 35.5, 45.4, 124.3, 150.0, 151.8.

(iii) Sodium cyanide (509 mg) in dimethyl sulfoxide (4.25 mL) was added to 4-(6-chlorohexyl)pyridine (1.71 g) dissolved in dimethyl sulfoxide (4.25 mL). The mixture was stirred at 60 °C for 3 h, poured into water (100 mL), and extracted with chloroform (3 × 15 mL). The combined extracts were washed with water (2 × 20 mL) and dried (Na_2SO_4) and the solvent evaporated to give **33** (1.45 g, 95.1%): ^{1}H NMR ($CDCl_3$) δ 1.32−1.42 (m, 2H), 1.43−1.54 (m, 2H), 1.60−1.74 (m, 4H), 2.32 (t, J = 7 Hz, 2H), 2.57−2.7 (m, 2H), 7.1 (d, J = 6 Hz, 2H), 8.5 (d, J = 6 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 17.5, 25, 28, 28, 30, 35, 120, 124, 150, 152; m/e = 188.9 $(M + H)^+$.

Ethyl 7-Piperidin-4-ylheptanoate (34). (g) (i) 7-Pyridin-4-ylheptanenitrile (**33**) (1.45 g) was stirred at 60−70 °C for 72 h with a mixture of ethanol (3.6 mL) and concentrated aqueous hydrochloric acid (3.6 mL). The cooled reaction mixture was partitioned between water (20 mL) and chloroform (20 mL) and the separated organic phase dried (Na_2SO_4) and evaporated to yield crystalline 7-pyridin-4-ylheptanoic acid ethyl ester hydrochloride (1.17 g, 52.3%): ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J = 7 Hz, 3H), 1.38 (m, 4H) 1.60 (quintet, J = 7 Hz, 2H), 1.72 (quintet,J = 7 Hz, 2H), 2.28 (t, J = 7 Hz, 2H), 2.90 (t, J = 7 Hz, 2H), 4.12 (q, J = 7 Hz, 2H), 7.74 (d J = 6 Hz, 2H), 8.74 (d, J = 7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14, 25, 28, 30, 34, 36, 60, 127, 140, 164, 174; m/e = 235.9 $(M + H)^+$.

(ii) 7-Pyridin-4-ylheptanoic acid ethyl ester hydrochloride (1.17 g) was dissolved in methanol (22.5 mL) and acetic acid (2.5 mL) and hydrogenated (1 bar) in the presence of Adam's catalyst (100 mg) at 20 °C for 16 h. The catalyst was removed by filtration and the solvents were evaporated to give **34** (1.13 g, 95%) which was stored in vacuo (NaOH pellets): ^{1}H NMR ($CDCl_3$) δ 1.23 (t, 3H), 1.3 (br.m, 8H), 1.42−1.7 (m, 5H), 1.9 (d, J = 14 Hz, 2H), 2.27 (quintet, J = 7 Hz, 2H), 2.78−2.9 (m, 2H), 3.42−3.5 (m, 2H), 4.13 (q, J = 7 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 14.6, 25.2, 26.6, 29.1, 29.3, 29.6, 34.4, 34.6, 36.0, 44.5, 60.5, 174.2; m/e = 241.9 $(M + H)^+$.

5-Guanidino-2(*S*)-[3-(1-methyl-1-phenylethyl)benzenesulfonylamino]pentanoyl Chloride Hydrochloride Salt (35). (a) (*S*)-Arginine (12.6 g) and potassium carbonate (12.03 g) were suspended in 50% water/dioxane (250 mL) with vigorous stirring and the reaction mixture cooled to <5 °C. 3-(1-Methyl-1-phenylethyl)benzenesulfonyl chloride (21.4 g) was added in 6 portions over 30 min at <5 °C; the mixture stirred at 20 °C for 2 h. The dioxane was removed by evaporation; the aqueous residue was acidified with concentrated aqueous hydrochloric acid and extracted with ethyl acetate (3 × 50 mL). The aqueous phase and all solid materials were combined and neutralized with aqueous sodium hydroxide (4 M). The resultant suspension was stirred for 16 h at 20 °C and the solid precipitate collected by filtration and stirred for 16 h with a little water. The solid was collected by filtration and dried (P_2O_5) to give 5-guanidino-2(*S*)-[3-(1-methyl-1-phenylethyl)benzenesulfonylamino]pentanoic acid (26 g, 83%): mp = 122−124 °C; TLC R_f = 0.1 ($CHCl_3$/MeOH/AcOH, 6:1:1), 0.36 (*n*-butanol/AcOH/water, 10:1:3); ^{1}H NMR (MeOH-d_4) δ 1.52−1.80 (m, 4H), 1.67 (s, 6H), 3.12 (t, J = 7 Hz, 2H), 3.30 (m, 1H), 3.50 (m, 1H), 7.15 (m, 1H), 7.2−7.27 (m, 4H), 7.33−7.42 (m, 2H), 7.62−7.7 (d, J = 6 Hz, 1H), 7.80 (s, 1H); ^{13}C NMR (MeOH-d_4) δ 26.3, 31.5, 32.5, 42.5, 44.8, 59.2, 126.1, 126.5, 127.5, 128.4, 129.8, 130.4, 133.3, 141.7, 151.5, 154.1, 159.1, 177.8; m/e = 433.1 $(M + H)^+$. Anal. ($C_{21}H_{28}N_4O_4S\cdot 2.3H_2O$) C, H, N, S.

(b) 5-Guanidino-2(*S*)-[3-(1-methyl-1-phenylethyl)benzenesulfonylamino]pentanoic acid (936 mg) was stirred with thionyl chloride (5 mL) for 2 h at 20 °C. Dry ether (40 mL) was added with vigorous stirring and the supernatant liquid decanted from the white gum formed which was then triturated with portions (2 × 40 mL) of dry ether. The gum was kept in vacuo (NaOH pellets) for 20 min to give a crisp white foam of **35** (0.94 g, 97%) which was used without further purification.

***N*-[4-Guanidino-1(*S*)-(2(*R*)-hydroxymethylpyrrolidin-1-ylcarbonyl)butyl]-3-(1-methyl-1-phenylethyl)benzenesulfonamide (38a). (c)** Pyrrolidin-2(*R*)-ylmethanol (204 mg) and triethylamine (0.28 mL) were dissolved in DMF (5 mL) and cooled in ice. 5-Guanidino-2(*S*)-[3-(1-methyl-1-phenylethyl)benzenesulfonylamino]pentanoyl chloride hydrochloride salt (**35**) (953 mg) dissolved in DMF (2 mL) was added dropwise during 10 min with stirring below 5 °C (pH > 9). After stirring for a further 30 min, the mixture was filtered, the filtrate evaporated to dryness, and the residual oil freed of DMF by coevaporation of the residue with ethanol (2 × 5 mL). The resultant oil was dissolved in methanol (2 mL) and added dropwise to vigorously stirred Na-dried ether (25 mL), the supernatant liquid decanted, and the residual gum held in vacuo (concentrated sulfuric acid) to give a crisp foam (1.09 g, 100%). The product was isolated from the foam by preparative high-pressure liquid chromatography (HPLC) (Zorbax C8) using acetonitrile:water:acetic acid (330:670:1 by vol.) to give 100 mg (9.2%) of product which was further purified by dissolution in a mixture of acetic acid (M, 3 mL) and aqueous hydrochloric acid (M, 2 mL) by passage through a column (40 × 3 cm) of Biogel-P2 resin, eluting with aqueous acetic acid (M) to give the hydrochloride salt of **38a** as lyophilized material: mp = 103−106 °C. Anal. ($C_{26}H_{37}N_5O_4S\cdot HCl\cdot H_2O$) C, H, N, S, Cl.

Compounds **38b,c** were prepared by a procedure analogous to **38a**.

***N*-[4-Guanidino-1(*S*)-(tetrahydroisoquinolin-1-ylcarbonyl)butyl]-1-(3-methyltetrahydroquinolyl)sulfonamide (39b). (a)** (*S*)-Arginine (12.8 g, 73.6 mmol) and potassium carbonate (12.2 g, 88.2 mmol) were dissolved in 50% water/dioxane (250 mL) with vigorous stirring, the reaction mixture cooled to <5 °C, and 3-methylquinolin-8-ylsulfonyl chloride (17.74 g, 73.6 mmol) added in 6 portions over 30 min at <5 °C. The mixture was stirred at 20 °C for 2 h. The reaction mixture was concentrated; the oily residue was gradually acidified with concentrated aqueous hydrochloric acid and extracted with chloroform (2 × 125 mL) and ethyl acetate (2 × 10 mL). The aqueous phase and all solid materials were combined, neutralized with aqueous sodium hydroxide (4 M), treated with saturated sodium bicarbonate solution, and concentrated to approximately half the original volume. The solid was collected by filtration, resuspended in a little water, stirred for 20 h at 20 °C, filtered, and dried (NaOH pellets) to give 5-guanidino-2(*S*)-[1-(3-methylquinolyl)sulfonylamino]-pentanoic acid (16.7 g, 60%): mp = 260 °C. Anal. ($C_{16}H_{21}N_5O_4S$) C, H, N, S.

(b) A procedure analogous to that described for **38a** (part b) was carried out but using 5-guanidino-2(*S*)-[1-(3-methylquinolyl)sulfonylamino]pentanoic acid (0.778 g, 2 mmol) and thionyl chloride (5 mL), to yield the corresponding acid chloride hydrochloride salt, 5-guanidino-2(*S*)-[1-(3-methylquinolyl)sulfonylamino]pentanoyl chloride·HCl salt (**36**) (1.046 g).

(c) A procedure analogous to that described for **38a** (part c) was carried out but using 5-guanidino-2(*S*)-[1-(3-methylquinolyl)-sulfonylamino]pentanoyl chloride·HCl salt (1.046 g) and tetrahydroisoquinoline (0.267 g, 2 mmol) to yield *N*-[4-guanidino1(*S*)-(tetrahydroisoquinolin-1-ylcarbonyl)butyl]-1-(3-methylquinolyl)sulfonamide·HCl salt (1 g, 1.88 mmol, 94.2%).

(d) *N*-[4-Guanidino-1(*S*)-(tetrahydroisoquinolin-1-ylcarbonyl)butyl]-1-(3-methylquinolyl)sulfonamide·HCl salt (200 mg, 0.376 mmol) was dissolved in methanol and acetic acid (11 mL of 10:1), 10% Pd/C (173 mg) added, and hydrogenated for 28 h with stirring. The catalyst was filtered off and the filtrate concentrated to dryness to yield 183 mg (90.6%) of crude product, which was purified by preparative HPLC [Zorbax C_8,

CH_3CN, H_2O, TFA (450:550:1)] and the residue converted to the acetate form (Dowex 1-X8 acetate) in 50% methanol/water, concentrated, and freeze-dried to yield 41.4 mg of pure **39b**. Anal. ($C_{25}H_{34}N_6O_3S \cdot CH_3COOH \cdot H_2O$) C, H, N, S.

Compounds **39a,c** were prepared by a procedure analogous to **39b**.

3(*RS*)-Methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic Acid {4-Guanidino-1(*S*)-[4-(2-hydroxyethyl)piperidin-1-ylcarbonyl]butyl}amide (42b). (a) 5-(3-Nitroguanidino)-2(*S*)-*tert*-butoxycarbonylaminopentanoic acid (92.2 g) was dissolved in DMF (465 mL), NMM (31.9 mL) added, the mixture cooled to −15 °C, isobutyl chloroformate (38 mL) added, and the reaction mixture stirred at −15 °C for 15 min. Acetic acid 2-piperidin-4-ylethyl ester hydrochloride (17b) (60 g) was dissolved in DMF (465 mL) with NMM (31.9 mL) and the solution cooled to −15 °C. The two solutions were combined with stirring below −10 °C; stirring continued for 30 min at −10 °C and then at 20 °C for 2 h. The solvent was removed by evaporation and the residue dissolved in ethyl acetate (300 mL) and washed with portions (2 × 250 mL) of satd aqueous sodium bicarbonate, satd sodium chloride, 7% aqueous citric acid, and satd sodium chloride, dried (Na_2SO_4); the solvent was evaporated to yield 5-(3-nitroguanidino)-2(*S*)- *tert*-butoxycarbonylamino-1-[4-(2-acetoxyethyl)piperidin-1-yl]pentan-1-one (**40**) as a gum, 84.8 g (60.2%).

(b) 5-(3-Nitroguanidino)-2(*S*)- *tert*-butoxycarbonylamino-1-[4-(2-acetoxyethyl)piperidin-1-yl]pentan-1-one **(40)** (127.6 g) was dissolved in saturated hydrogen chloride in acetic acid (625 mL) and the reaction mixture stirred at 20 °C for 2.5 h. The solvent was evaporated and the product pumped for 20 h (over NaOH pellets) to give 2(*S*)-amino-5-(3-nitroguanidino)-1-[4-(2-acetoxyethyl)piperidin-1-yl]pentan-1-one hydrochloride (128 g): pure by TLC (^{n}BuOH/AcOH/H_2O, 10:1:3) but containing residual acetic acid.

(c) The above 2(*S*)-amino-5-(3-nitroguanidino)-1-[4-(2-acetoxyethyl)piperidin-1-yl]pentan-1-one hydrochloride (128 g) was suspended in dry DMF (1 L) and Huenig base (46.6 mL) added to give a solution at pH 9 which was cooled to between 0 and 5 °C (ice/salt). A further 46.6 mL of Huenig base was added followed by 3-methylquinolinyl-8-sulfonyl chloride (58.2 g) in 5 equal portions over 30 min and the reaction mixture stirred at 20 °C for 3 h. The solvent was removed by evaporation and the residue dissolved in dichloromethane (800 mL) and washed with 7% aqueous citric acid (800 mL) and water (2 × 800 mL), with the aqueous phase being back-extracted with dichloromethane. The combined organic extracts were dried (Na_2SO_4) and the solvent was evaporated to give a residue which was triturated with ether to give 3-methylquinoline-8-sulfonic acid {4-(3-nitroguanidino)-1(*S*)-[4-(2-acetoxyethyl)piperidin-1-ylcarbonyl]butyl}amide (**41**) (Ar = MQS and X-Pip derived from **17b**) as a solid which was dried in vacuo, 128.8 g (82% for the two steps): TLC R_f = 0.43 ($CHCl_3$/MeOH/AcOH, 88:7:5).

(d) 3-Methylquinoline-8-sulfonic acid {4-(3-nitroguanidino)-1(*S*)-[4-(2-acetoxyethyl)piperidine-1-ylcarbonyl]butyl}amide (29.2 g) was dissolved in methanol (200 mL) at 0 °C and aqueous sodium hydroxide (M, 101.2 mL) added. The mixture was stirred at 20 °C for 1 h and aqueous hydrochloric acid (M, 101.2 mL) added. The solvents were removed by evaporation and the residue was shaken with chloroform (100 mL). Solids were removed by filtration washing with chloroform (100 mL) and the combined chloroform extracts were purified in two equal batches by passage through a pad (500 g, 13 cm diameter) of silica gel (70−230 mesh) [eluent chloroform (3.5l) and methanol:chloroform (5:95 by vol.)], collecting the eluate in fractions (250 mL). Fractions 14−29 afforded, after evaporation, 3-methylquinoline-8-sulfonic acid {4-(3-nitroguanidino)-1(*S*)-[4-(2-hydroxyethyl)piperidin-1-ylcarbonyl]butyl}amide, 15.1 g (55.7%): TLC R_f = 0.42 ($CHCl_3$/MeOH/AcOH, 6:1:1), 0.45 (^{n}BuOH/AcOH/H_2O, 10:1:3); chromatographically pure >99.5% by HPLC t_R = 11.2 min (Nucleosil 10C_{18}, gradient elution: 10−90% MeCN in 0.1% aqueous TFA over 30 min).

(e) 3-Methylquinoline-8-sulfonic acid {4-(3-nitroguanidino)-1(*S*)-[4-(2-hydroxyethyl)piperidin-1-ylcarbonyl]butyl}amide (60 g) was dissolved in a mixture of ethanol (1.05 L) and acetic acid (226 mL) and hydrogenated (1 bar) in the presence of 10% palladium on charcoal (12.5 g) for 92 h at 20 °C. Removal of the catalyst and solvent gave the acetate salt (67 g) which was dissolved in 2-propanol (300 mL) and converted to the phosphate salt by the slow addition of phosphoric acid (7.06 mL, 12.3 g of 88%, sp. gr. 1.75) in 2-propanol (100 mL). Addition of ether (2 L) to this solution with vigorous stirring gave a solid which was washed with ether and dried to constant weight (63.8 g, 95.9%). A portion of this (44.5 g) was dissolved in water (84 mL) and passed through a column (350 mL) of Dowex 1 (chloride form) resin eluting with water (700 mL). The solution was rotary evaporated (at <30 °C) and the residue dried by addition and evaporation of ethanol (3 × 100 mL). The residue was then dissolved in methanol (50 mL) and added to vigorously stirred dry ether (1 L) and the precipitate collected, washed with ether, and dried to give **42b** as the hydrochloride salt (38.2 g): mp = 108−112 °C; TLC R_f = 0.28 (^{n}BuOH/AcOH/H_2O, 10:1:3), 0.59 (^{n}BuOH/AcOH/EtOAc/H_2O, 1:1:1:1), 0.66 (EtOAc/Pyr/AcOH/H_2O, 5:5:1:3), 0.11 ($CHCl_3$/MeOH/AcOH/H_2O, 80:20:3:3); HPLC purity = 98.5% (RP Nucleosil-10-C_{18}, 1.8 mL/min, l = 210, 260 nm, t_R = 6.03 min (MeCN/H_2O/TFA, 300:700:1), 18.0 and 19.7 min (diastereomeric ratio = 68:32) (MeCN/H_2O/TFA, 250:750:1)); ^{13}C NMR (DMSO-d_6) δ 18.4 (CH_3-THQS) 24.5, 25.1 (*CH*-Me), 29.7, 31.9 (*CH*-CH_2−CH_2OH), 45.2 (piperidine CH_2−CH_2−N), 47.6 (piperidine CH_2−N), 51.5 (Arg-a *CH*) 58.1 (CH_2OH), 113.5, 118.5, 122.1 (*C*-SO_2NH), 127.2, 133.4, 142.3 [HN-*C*(C)-C−SO_2NH], 157.1 [HN-*C*(=NH)-NH_2], 168.2 (CONH); $[\alpha]^{26}_D$ = +49.5 ± 0.7 (*c* 1.12, 0.2 M HCl), 121 ± 0.7 (*c* 1.14, MeOH). Anal. ($C_{23}H_{38}N_6O_4S \cdot HCl \cdot 4H_2O$) C, H, N, S, Cl.

Inhibitors **42a**, **42c**−**e**, and **45a**−**i** were prepared by a route analogous to that used for **42b**.

Malonic Acid Mono(2-{1-[5-guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}ethyl) Ester (43a). Chlorocarbonylacetic acid benzyl ester (2.8 g) and **42b** hydrochloride (1.5 g) were dissolved in dry pyridine (30 mL) and stirred with exclusion of moisture for 72 h at 20 °C. Solvent was removed by rotary evaporation and the residue purified by flash chromatography (chloroform/methanol/acetic acid, 6:1:1) to give the semisolid benzyl ester of malonic acid (2-{1-[5-guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}ethyl) ester hydrochloride salt (2 g) which was dissolved in a mixture of methanol (40 mL) and acetic acid (2 mL) and hydrogenated (1 bar) for 16 h at 20 °C in the presence of 10% palladium on charcoal (570 mg). The catalyst was removed by filtration through a pad of Celite and the solvents were removed to give a residue (1.75 g, 100%) which was purified by preparative HPLC (chloroform/methanol/trifluoroacetic acid, 390:610:1), to afford **43a** (0.23 g, 13%) as the trifluoracetate salt: TLC R_f = 0.32 ($CHCl_3$/MeOH/AcOH, 6:1:1); single peak by HPLC t_R = 5.0 min (Zorbax C_8, CH_3CN/H_2O/TFA, 390:610:1). Anal. ($C_{26}H_{40}N_6O_7S \cdot CF_3COOH \cdot 0.5H_2O$) C, H, N, S.

Pentanedioic Acid Mono(2-{1-[5-guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)-pentanoyl]piperidin-4-yl}ethyl) Ester (43b). Glutaric anhydride (2.3 g) and **38** (part b) (2.0 g) were dissolved in dry pyridine (40 mL) at −20 °C and stirred for 16 h at room temperature. The reaction mixture was purified as described for the analogous **39a** initially by flash chromatography to give the crude product (2.4 g, 97.6%) and then by HPLC to give **43b** (0.265 g, 9%) as the trifluoroacetate salt: TLC R_f = 0.36 ($CHCl_3$/MeOH/AcOH, 6:1:1); single peak by HPLC t_R = 8 min (Zorbax-C_8, CH_3CN/H_2O/TFA, 400:600:1). Anal. ($C_{28}H_{44}N_6O_7S \cdot CF_3COOH \cdot 0.5H_2O$) C, H, N.

***N*-[4-Guanidino-1(*S*)-(2(*R*)-hydroxymethylpiperidin-1-ylcarbonyl)butyl]-3-(1-methyl-1-phenylethyl)benzenesulfonamide (44).** Analogously as described for **42b** but using 3-(1-methyl-1-phenylethyl)benzenesulfonyl chloride and piperidine-2(*R*)-yl carboxylic acid ethyl ester in place of 3-methylquinoline-8-sulfonyl chloride and 3-piperidin-4-ylpropionic acid methyl ester was prepared [5-(3-nitroguanidino)-2(*S*)-(3-

(1-methyl-1-phenylethyl)benzenesulfonylamino)pentanoyl]piperidine-2(*R*)-carboxylic acid ethyl ester.

(g) [5-(3-Nitroguanidino)-2(*S*)-(3-(1-methyl-1-phenylethyl)-benzenesulfonylamino)pentanoyl]piperidine-2(*R*)-carboxylic acid ethyl ester (2.1 g) was dissolved in THF (40 mL) and heated to reflux. A solution of lithium borohydride (2 M) in tetrahydrofuran (3.4 mL) was added dropwise and the solution heated at reflux for a further 45 min and then cooled to room temperature. The mixture was cooled to 5−10 °C and water (15 mL) added dropwise. Solid potassium carbonate (5 g) was added and the mixture stirred, allowing to come to room temperature over 30 min. The THF was removed by evaporation and the aqueous residue extracted with ethyl acetate (2 × 25 mL). The combined extracts were washed with aqueous potassium carbonate solution (10% by wt., 15 mL) and satd aqueous sodium chloride solution (15 mL), dried ($MgSO_4$), filtered, and evaporated to give a foam which was purified by column chromatography on Kieselgel 40 using ethyl acetate: dichloromethane (3:7 then 1:1 then 7:3 by vol.) and finally acetone:ethyl acetate (1:1 by vol.) to obtain *N*-[4-(3-nitroguanidino)-1(*S*)-(2(*R*)-hydroxymethylpiperidin-1-ylcarbonyl)butyl]-3-(1-methyl-1-phenylethyl)benzenesulfonamide (1.32 g, 68.3%) as a foam.

(h) *N*-[4-(3-Nitroguanidino)-1(*S*)-(2(*R*)-hydroxymethylpiperidin-1-ylcarbonyl)butyl]-3-(1-methyl-1-phenylethyl)benzenesulfonamide was treated as described for **38**(part b) but using methanol only as the solvent for hydrogenation to give **44** as a white solid after lyophilization from aqueous solution: TLC R_f = 0.55 (BuOH/AcOH/H_2O, 3:1:1);^{13}C NMR (DMSO-d_6) δ 18.8, 24.6, 24.8, 25.3, 29.6, 30.1, 51.3, 54.1, 58.8, 123.7, 124.0, 124.3, 125.7, 126.3, 128.0, 128.5, 130.9, 140.8, 149.2, 151.2, 156.9, 169.3. Anal. ($C_{27}H_{39}N_5O_4S\cdot2.5H_2O$) C, H, N.

Acetic Acid 2-{1-[5-Guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}ethyl Ester (46). (k) To 3(*RS*)-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic acid {4-guanidino-1(*S*)-[4-(2-*tert*-butoxyethyl)piperidin-1-ylcarbonyl]butyl}amide (prepared in an analogous fashion to **42b** using piperidine intermediate **16h** (268 mg) in water (0.3 mL)) was added glacial acetic acid (3.5 mL), maintaining the temperature below 15 °C. When dissolution was complete, HBr in acetic acid (1.4 mL of 45% w/v) was added dropwise and the mixture stirred for 3.5 h. The mixture was concentrated to dryness; the residue was dissolved in chloroform (∼25 mL) and washed with ice-cold water (2 × 5 mL), satd Na_2CO_3 solution (2 × 5 mL), and satd NaCl (2 × 5 mL), dried ($MgSO_4$), evaporated to dryness, and then coevaporated with ether (×2) to yield **46**: MS(FAB) $[M+H]^+$ = 535. The ^{13}C NMR spectrum was consistent with the claimed structure.

3(*RS*)-Methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic Acid [4-Guanidino-1(*S*)-(2(*R*)-hydroxymethylpiperidin-1-ylcarbonyl)butyl]amide (47a). Prepared in an analogous way to that described for **42b** but using 2(*R*)-*tert*-butoxymethylpiperidine (**16b**) in place of **7b** and achieving selective removal of the Boc group from the arginyl piperidine intermediate (at 0.04 M dilution) using 1 equiv of hydrogen chloride in acetic acid/dichloromethane (1:2 by volume) at 0 °C for 45 min. The final removal of the *tert*-butyl group and purification were carried out by the following procedure: 3(*RS*)-Methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic acid [4-guanidino-1(*S*)-(2(*R*)-*tert* -butoxymethylpiperidin-1-ylcarbonyl)butyl]amide (2.68 g) was suspended in water (3 mL), glacial acetic acid (35 mL) added, and the mixture stirred at 15 °C until all had dissolved. 45% HBr in acetic acid (14 mL) was added dropwise while maintaining the temperature at 15 °C; the mixture stirred for 3.5 h and evaporated to dryness under high vacuum. The residue was suspended in dioxane (50 mL), concd NH_4OH/H_2O added at 20 °C until the pH = 10.5−11, and stirring continued for 18 h. Solid Na_2CO_3 (10 g) was added, the dioxane was removed under high vacuum, the aqueous residue was extracted with $CHCl_3$ (3 × 75 mL), the combined organics were washed with satd Na_2CO_3 solution (2 × 50 mL) and satd NaCl (2 × 50 mL), and dried ($MgSO_4$), filtered, and evaporated. The residue was stirred with isopropyl alcohol (75 mL), all insoluble material filtered off, ether (300 mL) added with vigorous stirring, and the precipitated solid filtered and washed with ether. This procedure of precipitation and ether washing was repeated and the resultant solid dried under high vacuum to yield 1.3 g (54.2%) of **47a** as a white solid: TLC R_f = 0.54 (BuOH/AcOH/H_2O, 3:1:1); ^{13}C NMR (DMSO-d_6) δ 18.3, 18.8, 24.5, 25.0, 29.7, 47.5, 49.6, 51.1, 51.6, 54.2, 57.5, 58.8, 113.5, 119.2, 122.0, 122.2, 127.0, 133.3, 142.0, 156.9, 169.4, 169.5. Anal. ($C_{22}H_{36}N_6O_4S\cdot2.5H_2O$) C, H, N.

Inhibitors **47b**−**d** were prepared analogously to **47a** but using the respective piperidines.

3(*RS*)-Methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic Acid [4-Guanidino-1(*S*)-(3(*R*)-hydroxymethylpiperidin-1-ylcarbonyl)butyl]amide (47e). Analogously as described for **43a**, except using 3(*R*)-*tert*-butoxymethylpiperidine (**16d**) and a different final acidolysis procedure: 3(*RS*)-methyl-1,2,3,4-tetrahydroquinoline-8-sulfonic acid [4-guanidino-1(*S*)-(3(*R*)-*tert*-butoxymethylpiperidin-1-ylcarbonyl)butyl]amide (798 mg) was dissolved in trifluoroacetic acid (20 mL), stirred, and cooled to −5 °C and a stream of hydrogen bromide bubbled into the solution for 1 h. The flask containing the mixture was stoppered and kept at 20 °C for 2 h. The mixture was concentrated under vacuum and the residue triturated with ether to give an off-white solid which was coevaporated (twice) with ether. The solid was dissolved in a mixture of water (10 mL) and dioxane (5 mL); the solution was adjusted to pH > 10 by addition of ammonia (sp. gr. 0.880) and extracted with ethyl acetate (3 × 20 mL). The combined extracts were dried ($MgSO_4$) and evaporated, and the residue obtained was triturated with ether to give a solid which was collected by filtration, washed with ether, and dried in vacuo to give **47e**: mp = 0.60 °C; ^{13}C NMR (DMSO-d_6) δ 18.4, 24.1, 24.4, 24.9, 25.1, 26.6, 29.6, 35.3, 45.2, 45.5, 47.6, 48.3, 51.3, 51.7, 63.3, 63.4, 113.3, 113.4, 118.4, 118.6, 118.8, 122.0, 122.2, 122.4, 127.1, 133.3, 142.2, 157.0, 168.1, 168.2, 168.4.

Inhibitors **47f** and **47g** were prepared analogously to **47e**.

{1-[5-Guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}acetic Acid (48b). (a) Analogously as described for **42b** (parts a−c, e), but using piperidin-4-ylacetic acid ethyl ester trifluoroacetate (**28**) was prepared {1-[5-guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}acetic acid ethyl ester as a pale yellow foam: mp = 86−95 °C.

(d) Analogously as described for the saponification step (part d) for **42b** but using {1-[5-guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}acetic acid ethyl ester (0.5 g) was prepared **48b** (108 mg, 23%): mp = 183−189 °C; TLC R_f = 0.55 (BuOH/AcOH/H_2O, 3:1:1); ^{13}C NMR (DMSO-d_6) δ 18.3, 24.3, 25.1, 29.7, 33.3, 35.4, 36.6, 44.7, 44.9, 47.7, 51.6, 113.5, 113.6, 118.7, 122.0, 122.2, 127.2, 133.3, 142.2, 157.5[NH−C(=NH)NH_2], 168 (CONH), 176.8(COOH). Anal. ($C_{22}H_{37}N_6O_5S\cdot H_2O$) C, H, N.

Inhibitor **48d** was prepared as for **45b** but using piperidine **9b** and then carrying out the saponification as for **42b**.

3-(3-{1-[5-Guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]-piperidin-4-yl}propionylamino)propionic Acid (49). 3-{1-[5-Guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}propionic acid (**48c**) (200 mg) and 3-aminopropionic acid ethyl ester hydrochloride (50 mg) were added to a solution of triethylamine (90 mL) in DMF (1.5 mL) and stirred with DCC (75 mg) and HOBt·H_2O (50 mg) at 20 °C for 3 h. A further portion of DCC (75 mg) was added and the mixture stirred for 72 h at 20 °C. The mixture was filtered, the filtrate evaporated to dryness, and the residue purified by flash chromatography ($CHCl_3$/MeOH/AcOH, 6:1:1, R_f = 0.27) to give the pure ethyl ester (200 mg, 94%). This was dissolved in methanol (2 mL), aqueous sodium hydroxide (M, 1.3 mL) added, and the mixture stirred at 20 °C for 16 h. Aqueous sulfuric acid (0.5 M, 1.3 mL) was added and the solution evaporated to dryness. The residue was kept in vacuo (NaOH pellets) for 16 h and then extracted with portions (3 × 10 mL)

of hot ethanol. Evaporation of the combined extracts by lyophilization from dilute acetic acid solution gave the stable solid acetate salt **49** (100 mg, 47.6%): TLC $R_f = 0.18$ ($CHCl_3$/MeOH/AcOH, 6:1:1); 1 main peak by HPLC [Zorbax C_8, gradient elution (35 min) CH_3CN/H_2O/TFA, 0:1000:1 to 950:50:1]. Anal. ($C_{27}H_{43}N_7O_6S \cdot 2CH_3COOH \cdot 0.5H_2O$) C, H, N, S.

[2-(2-{1-[5-Guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}ethylcarbamoyl)ethyl]phosphonic Acid (50). Analogously as described for **42b** (steps a−c) but using [2-(2-piperidin-4-ylethylcarbamoyl)ethyl]phosphonic acid diethyl ester acetate salt (**27c**) (513 mg) in place of acetic acid 2-piperidin-4-ylethyl ester hydrochloride was prepared [2-(2-{1-[5-guanidino-2(*S*)-(3(*RS*)-methyl-1,2,3,4-tetrahydroquinolin-8-ylsulfonylamino)pentanoyl]piperidin-4-yl}ethylcarbamoyl)-ethyl]phosphonic acid diethyl ester which was stirred in saturated hydrogen bromide in acetic acid (5 mL) at 20 °C for 16 h. The solvent was removed by rotary evaporation and the oily residue obtained triturated with dry ether to afford a solid which was purified by gel filtration chromatography to give **50** as a solid after lyophilization (165 mg, 35%). Anal. ($C_{26}H_{44}N_7O_7SP \cdot 2.5H_2O$) C, H, N, S, P.

Supporting Information Available: Tables of experimental data. This material is available free of charge via the Internet at http://pubs.acs.org.

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