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Vinyl Sulfone-based Peptidomimetics as Anti-Trypanosomal Agents: Design, Synthesis, Biological and Computational Evaluation.

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Abstract A series of vinyl sulfone-containing peptidomimetics were rationally designed, synthesized and evaluated against *Trypanosoma brucei brucei*. These electrophilic compounds are likely to exert their antitrypanosomal activity *via* inhibition of trypanosomal cysteine proteases, TbCatB and rhodesain, through alkylation of a key cysteine residue within the protease active site. The series was designed to present complementary groups to naturally recognized peptide substrates, whilst probing tolerance to a range of substitutions at the P1, P1' and P2 positions. The most potent compound, **29** (EC_{50} = 70 nM, *T.b.brucei* whole cell assay) displayed minimal toxicity (>785 times selectivity) when assayed for cytotoxicity against the human promyelocytic leukemia (HL-60) cell line. Cells treated with compound **29**, as with K777 (**2**), exhibited an increase in both the number of multinucleated cells and cells with swollen flagellar pockets. Computational analysis revealed a strong correlation between the hypothetical binding mode in TbCatB/rhodesain and trypanocidal activity *in vitro*.

1. Introduction*

African sleeping sickness, or African trypanosomiasis, is a parasitic disease endemic in over 36 countries in sub-Saharan Africa.[1,2] It is caused by the flagellate kinetoplastid protozoan parasite, *Trypanosoma brucei,* which resides extracellularly in the blood and tissue fluids of the mammalian host and is transmitted by the tsetse fly. *Trypanosoma brucei gambiense* is widespread in central and western Africa whereas *Trypanosoma brucei rhodesiense* is restricted to east and east central Africa and is less common.[1,2] The colloquial term for African trypanosomiasis - African sleeping sickness - refers to the most serious phase of the disease which occurs when the parasite penetrates the central nervous system causing a sense of confusion, changes in behaviour, poor coordination and a disturbed sleep cycle. It is currently estimated that between 30,000 and 50,000 deaths per

*Abbreviations used: Cbz: benzyloxycarbonyl; Bn: benzyl; *i*-Bu: isobutyl; Boc: *tert*-butyloxycarbonyl; EDCI: *N*-(3,dimethylaminopropyl)-*N*'-ethylcarbodiimide; DAPI: 4',6-diamidino-2-phenylindole; DIPEA: diisopropylethylamine; DCM: dichloromethane; HL-60: human promyelocytic leukemia cells; THF: tetrahydrofuran; PCC: pyridinium chlorochromate; EWG: electron withdrawing group; Ph: phenyl; TFA: trifluoroacetic acid.

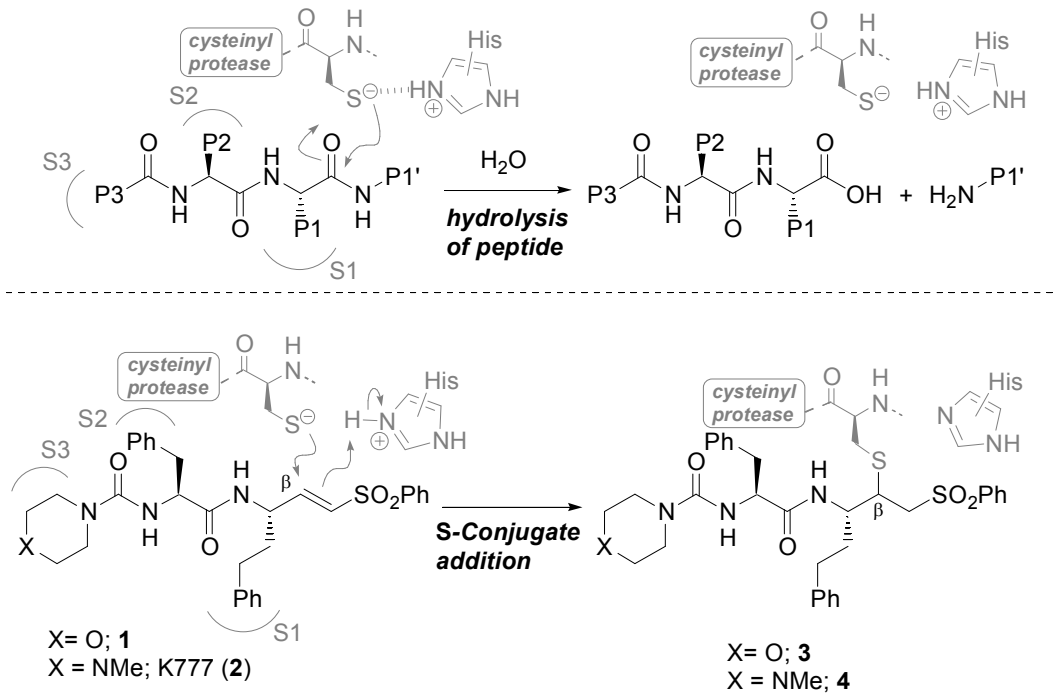
year result from this disease.[1-3] In addition, several subspecies (*Trypanosoma brucei brucei*, *Trypanosoma congolense* and *Trypanosoma vivax*) cause Nagana (a Zulu word meaning "to be depressed"), the bovine equivalent of African trypanosomiasis. In Africa, it has been estimated that if the tsetse fly could be eradicated, or an effective, cheap treatment were available, a doubling in cattle numbers could be achieved that would yield an extra annual income of US $5 billion.[4]

In the absence of medical intervention sleeping sickness is ultimately fatal. Current treatment for human sleeping sickness still relies heavily on drugs developed many years ago, all of which are toxic, show decreasing efficacy and suffer from administration/patient compliance issues.[5] Based on these points, new strategies for the control and treatment of African sleeping sickness and Nagana could potentially benefit many people.[1-5]

Cysteine proteases present in *T. brucei* play an essential role to the parasite's life cycle and as such represent a potential target for pharmaceutical intervention.[6-9] These proteins belong to a wide class of proteolytic enzymes, that rely on a nucleophilic cysteine residue within the active site, to assist in peptide bond hydrolysis (Scheme 1).[6] They have been classified into three structurally distinct groups: papain-like (Clan CA), interleukin-1β-converting-like (Clan CD) and picornain-like (Clan PA). Clan CA, papain-like, cysteine proteases represent the largest group and are widely expressed in both plants and animals and are essential for the life cycle of several parasites, including *trypanosoma*.[6-9]

Of the several types of compound developed and reported as parasitic cysteine protease inhibitors most rely on covalent derivatization of the key cysteine residue. For example, peptide-based aldehydes, α-halomethyl ketones, nitriles, epoxides, aziridines and Michael-type acceptors have all been reported as effective inhibitors.[6-12] Typically, these inhibitors have a peptide component (or mimic) that binds optimally to the S1 to S3 subsite of the protease. Additionally, electrophilic

functionality with the ability to react with and derivatize the nucleophilic sulfur atom, either reversibly, or irreversibly, is included (see, for example, Scheme 1).



Scheme 1. Cysteine protease-based peptide hydrolysis and the mechanism-based cysteine protease inhibition by vinyl sulfones **1** and **2**.

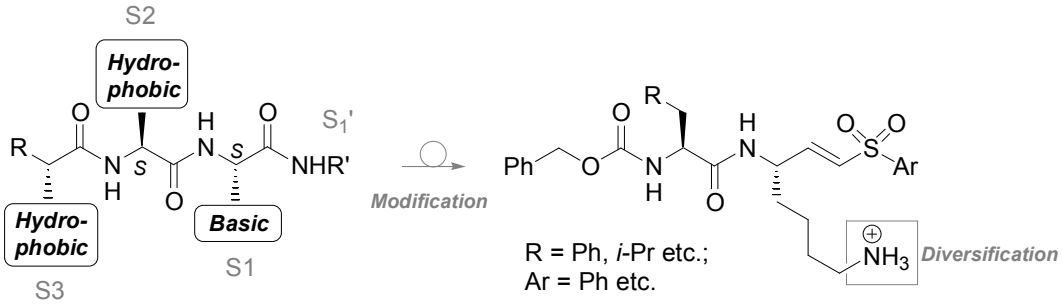
One such class of inhibitor; peptidyl vinyl sulfones, was first introduced by Hanzlik and co-workers as a group of compounds which inhibit the cysteine protease, papain.[12,13] Linked to the likely mechanism of action, the *S*-conjugate reaction of vinyl sulfones (as shown in Scheme 1) with sulfur-based nucleophiles (particularly under basic conditions) is a reliable chemical process. Palmer *et al*. expanded on these studies synthesizing a library of peptidyl vinyl sulfones that contain "functional scaffolds," combining inhibitor-protease complementarity in terms of binding with enzymatic deactivation.[14] In this important study, it was shown that vinyl sulfones specifically inhibit cysteine proteases over serine proteases (despite the fact that the serine proteases often possess a similar substrate profile, in terms of the type of amino acid side-chain preferentially recognized). The irreversibility of cysteine protease inactivation by vinyl sulfones was also

explored and it was shown that cathepsin B was completely inactivated by **1**, which following purification by column chromatography demonstrated no recovery of enzymatic activity. Finally, in terms of selectivity and possible non-discriminate toxicity, the stability of selected vinyl sulfones toward the ubiquitous tripeptide glutathione was explored. Pre-incubation of vinyl sulfone **1** with glutathione for 22 hours showed no subsequent loss of inhibitory potency, suggesting selectivity for the protease.[14,15]

Rosenthal and colleagues showed in 1993 that a peptidyl α-fluoromethyl ketone cured 80% of malaria-infected mice when it was administered subcutaneously over 4 days. Unfortunately however, the high doses required to cure malarial infection resulted in significant toxic effects to the host.[16] Subsequently, the same group revealed that a peptidyl vinyl sulfone was a potent inhibitor of falcipain, a cysteine protease found in *Plasmodium falciparum*.[17] This compound markedly delayed the progression of malaria and cured about 40% of mice when they were treated orally twice-a-day for four days without the associated toxicity with the previous study. In 1998, McKerrow *et al*. reported that dipeptide vinyl sulfone **2** rescued mice from a lethal *Trypanosoma cruzi* infection as a result of the inhibition of the cysteine protease, cruzain.[18] Now known as K777 (also K11777), it was subsequently shown that this vinyl sulfone **2** protected Beagle dogs from cardiac damage during infection by *T. cruzi*.[19] In 2009 the results of a preclinical trial were reported: K777 proved to be non-mutagenic, well-tolerated, and demonstrated efficacy in models of acute and chronic Chagas' disease in both mice and dogs.[20] Since 2007, FDA approval has been sought to allow K777 to enter Phase I trials in humans.[20]

Specifically for African trypanosomiasis, in 1999 it was reported that a peptide-based, diazomethyl ketone, killed bloodstream forms of *T. brucei in vitro* and *in vivo*.[21] Although, after cessation of treatment the effect proved not to be permanent, infected mice treated with the inhibitor survived nearly twice as long as those treated with placebo.[21] Importantly, the trypanocidal action of the

compound was associated with almost complete inhibition of brucipain (also known as rhodesain, trypanopain-tb), the cysteine protease in *T. brucei*. Coetzer also evaluated a range of α-electrophilic ketone, and vinyl sulfone derivatives for the bloodstream form of *Trypanosoma brucei brucei*. Although *T. b. brucei* causes Nagana, it is also a suitable model for *T. b. gambiense* and *T. b. rhodesiense*.[4] The ketone derivatives were moderately potent, with the most potent compound in the study being vinyl sulfone-based, in particular **2** (IC_{50} 0.1 μM).[4] In an effort to identify the possible intracellular targets of these inhibitors, biotinylated derivatives were incubated with live and/or lysed *T. b. brucei*. The results demonstrated that the major intracellular target was trypanopain-Tb, a key lysosomal cysteine proteinase.[4] In several publications, the type of peptide recognized and digested by the papain-like cysteine proteases presented by *T.b. rhodesiense* and *T. cruzi* have been profiled using fluorogenic tagged peptides and microarray techniques.[9,26] Summarizing this data, it can be concluded that both enzymes prefer basic, lysine and arginine residues in the S1 position, and bulky, hydrophobic residues in S2 (see Schemes 1 and 2). Rhodesain equally accepted leucine and phenylalanine whereas cruzain prefers phenylalanine. Stereochemistry was also interrogated and a preference for the naturally occurring amino acid stereogenicity was demonstrated.[26a] Noteworthy, the human cysteine protease; cathepsin L, shares many similarities with these parasitic enzymes, both in terms of substrate specificity and degree of amino acid homology.[26b] In 2009 the first crystal structure of rhodesain bound to **2** was published providing additional understanding of the substrate-binding site profile.[20]



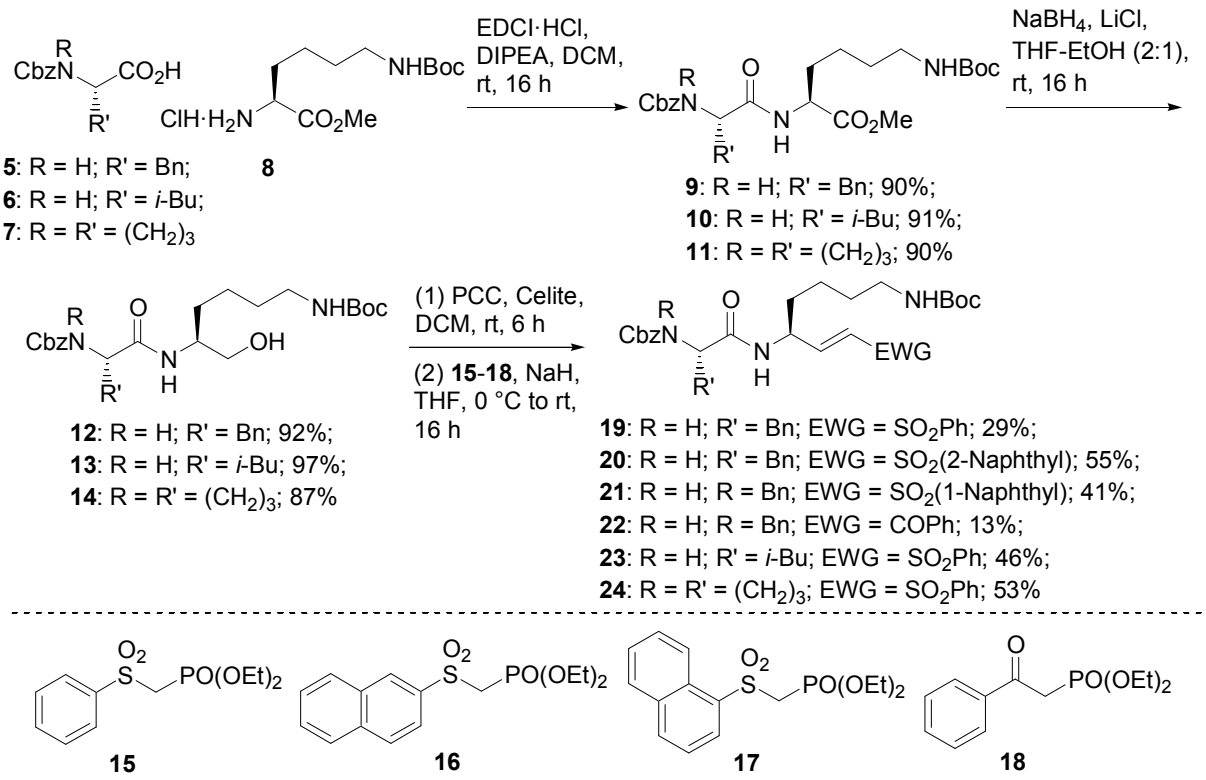
Scheme 2. Trypanosome cysteine protease substrate specificity and lysine-based vinyl sulfones inhibitors.

Based on the clear preference for a basic residue in the parasitic enzyme binding pocket (S1) and the demonstration that vinyl sulfones are an efficacious, biologically active scaffold upon which to base irreversible inhibitor design[27], we felt that appropriately designed inhibitors of this type would be of interest (Scheme 2). To the best of our knowledge only one example of a lysine-vinyl sulfone[4] and one example of an arginine-vinyl sulfone[28] based cysteine protease inhibitor have been reported. In addition, it was also envisaged that this basic group would represent a functional handle, in order to both explore the S1 subsite and also to tune physiochemical properties. Thus, a series of lysine-containing dipeptide vinyl sulfones were synthesised, assessed for trypanocidal activity and toxicity, and finally rationalised *via* novel computational methods.

Results and discussion

Chemistry

As outlined in Scheme 3, using commercially available protected L-lysine **8** peptide bond formation, mediated by the water soluble carbodiimide EDCI[29] was efficiently achieved with Cbz-protected L-phenylalanine **5**, L-leucine **6** and L-proline **7**. On treatment with lithium borohydride[30] the resultant dipeptides **9** to **11** were efficiently converted to their corresponding primary alcohols **12** to **14**. The next task was an alcohol to aldehyde oxidation, followed by an olefination process. In our hands, Swern oxidation (including its variants) and Dess-Martin periodinane led to poor conversion and the formation of additional reaction products. Eventually it was found that pyridinium chlorochromate (PCC) in the presence of Celite, to facilitate product retrieval from the chromium by-products, proved optimal.[31] Using these conditions the Phe-Lys aldehyde (not shown) could be reproducibly isolated in approximately 50% yield, after purification by flash column chromatography.



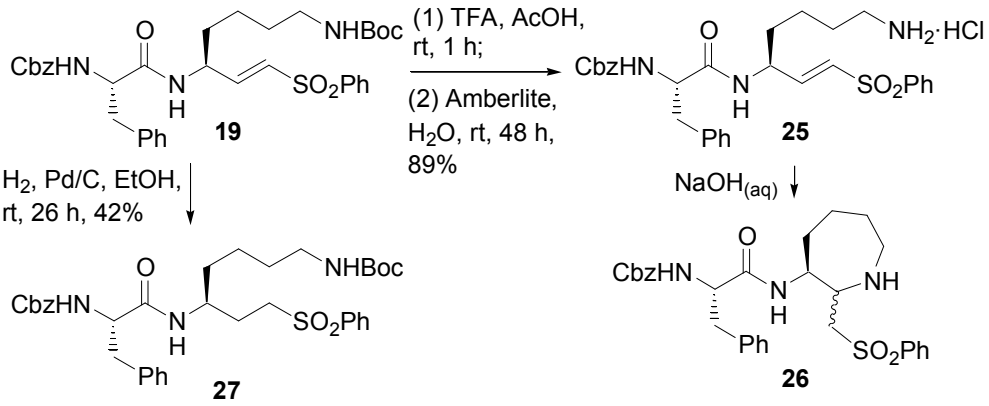
Scheme 3. Synthesis of dipeptide vinyl sulfones.

This material could then be converted into the corresponding vinyl sulfone **19** following Horner-Wadsworth-Emmons olefination[32] using the sodium salt of phosphonate **15** (for the synthesis of phosphonates **15**-**18** see electronic supporting information). As anticipated, good *trans*-selectivity was observed and **19** was isolated in 52% yield (29% over two steps). The likely explanation for the modest yield observed over these two steps is associated with the instability of the aldehyde.[33] Consequently, subsequent attempts circumvented its purification and under these conditions the crude aldehyde could be more efficiently converted into the 2- and 1-naphthyl vinyl sulfones **20** and **21** in 55 and 41% isolated yields respectively. In order to compare activity of the vinyl sulfone-based inhibitors with the more electrophilic α,β-unsaturated ketone, the synthesis of **22** was attempted. However, even under the optimized procedure, only small amounts of **22** could be

isolated. Linked to a desire to probe effects associated with the identity and conformation of the second amino acid, leucine **23** and proline **24** based vinyl sulfones were similarly accessed.

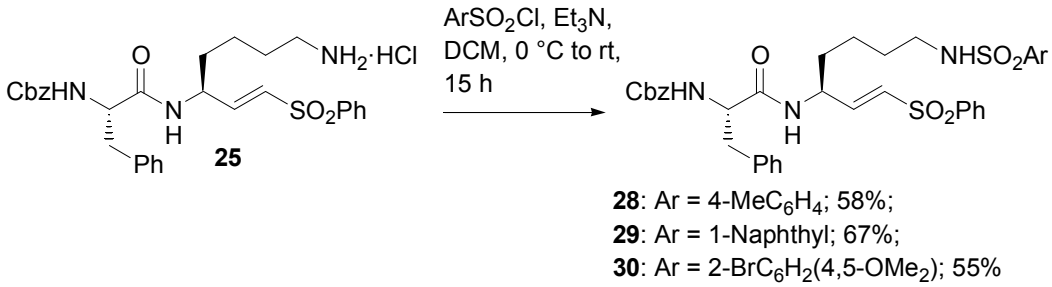
As mentioned in the introduction, based on the substrate specificity we were interested in accessing the compound in which a free amino group was present on the lysine side-chain (Scheme 2). Thus, the *tert*-butyloxycarbonyl (Boc) group in vinyl sulfone **19** was chemoselectively removed on treatment with trifluoroacetic acid (TFA) in acetic acid (Scheme 4). This process efficiently generated the corresponding ammonium salt as its trifluoroacetate salt. Associated with characterization and improved solubility, using hydrochloric acid treated Amberlite resin, the counterion was exchanged with a chloride, according to a literature procedure,[34] in order to generate **25**. This compound was stored and handled as the ammonium salt since upon adjustment of the pH proton NMR indicated that the vinyl group was substantially lost. Mass spectrometry of this basified material, however, indicated the same molecular ion. We attributed this data to an intramolecular conjugate addition process generating azepine **26**, presumably as a mixture of diastereoisomers.

Since the vinyl sulfone inhibitors were designed to alkylate the catalytically active cysteine residue in the cysteine protease peptide binding pocket, it was of interest to investigate how removal of the potential for this mode of action impacted on the overall biological activity of the dipeptide compound.



Scheme 4. The Boc-removal and hydrogenation of **19**.

To this end, alkene hydrogenation of **19** was performed in order to convert the vinyl sulfone into its saturated counterpart. In relation to this conversion, it is notable that under the reaction conditions alkene-hydrogenation occurs more rapidly than hydrogenolysis of the Cbz group and thus **27** may be isolated directly in reasonable yield.



Scheme 5. *N*-Derivatization of ammonium salt **25**.

The efficient preparation of **25** also presented the possibility of structural diversification by elaboration of the amino group, however, if this were to prove successful the desired intermolecular process would need to proceed at a faster rate than the intramolecular conjugate addition (forming **26**). As shown in Scheme 5, it did indeed prove possible to convert the ammonium salt in **25** directly to the corresponding sulfonamides **28**, **29** and **30** in moderate yield with base in the presence of the corresponding sulfonyl chlorides.

Biochemistry

The effect of compounds **19-25** and **27-30** on the viability of *in vitro* cultures of *Trypanosoma brucei brucei* were tested and compared with a known cysteine protease inhibitor, K777 (**2**), currently being developed as an anti-trypanosomal therapy for Chagas' disease.[35] Parasite-mediated reduction of Alamar-Blue was used as an indicator of cell viability. A dose-dependent effect on trypanosome viability was observed with EC_{50}'s ranging from 0.07-26.4 μM for the series, which compared favourably with the EC_{50} of 5.56 μM obtained for **2** (Figure 1). Additionally, the

compounds exhibited a >2-fold selective inhibition of parasite versus mammalian cell (HL-60 cell line) proliferation *in vitro* (Figure 2), with the most selective compound, **29**, being >785 times more potent for *T.b.brucei* (Table 1). Within the series the only significant effect on HL-60 cells was evident at micromolar concentration with the α,β-unsaturated ketone **22**. Nwaka and Hudson recently recommended a selectivity index of >100 to pursue animal studies[36] in the Special Programme for Research and Training in Tropical Diseases at the World Health Organization (WHO/TDR) pointing to compound **29** potentially being an excellent candidate for further studies.

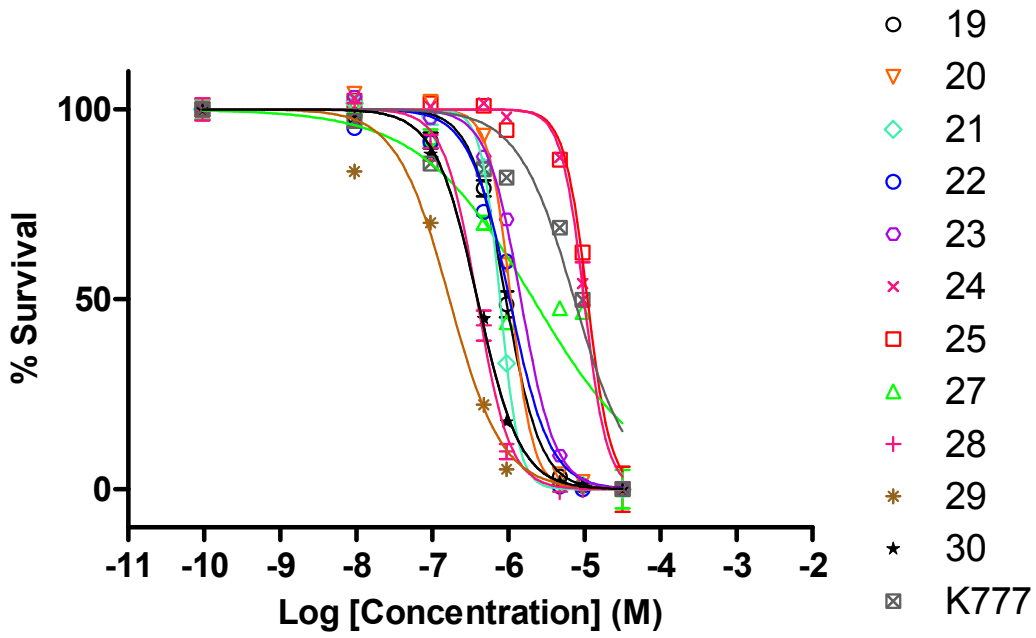


Figure 1. Response (4-Parameter logistic non-linear regression) of *Trypanosoma brucei brucei* to compounds **19-25**, **27-30**. Cells (2 x 10^3/well) were incubated in the presence of inhibitors for 24 h and Alamar-Blue added approximately 18 h before determining percentage inhibition of the fluorescent signal compared to controls minus inhibitor.

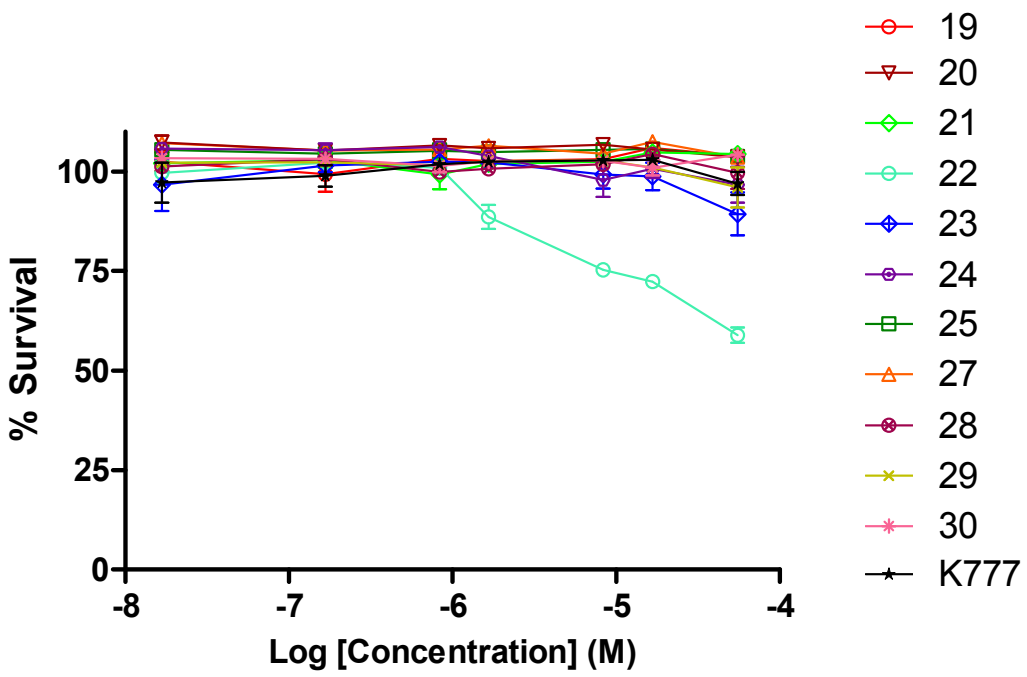
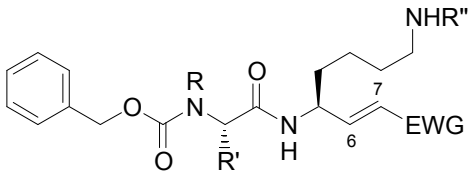


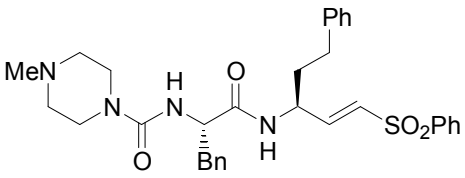
Figure 2. Response of human promyelocytic leukemia cells (HL-60) to compounds **19-25**, **27-30**. Cells (2 x 10^3/well) were incubated in the presence of inhibitors for 48 h and Alamar-Blue added approximately 18 h before determining percentage inhibition of the fluorescent signal compared to controls minus inhibitor.

Generally, all compounds exhibited the ability to perturb *Trypanosoma brucei brucei* growth versus controls, however, in terms of relating structure to biological behaviour several aspects associated with their efficacy are evident. Linked to the mechanism-based action of these cysteine protease inhibitors (Scheme 1); comparing the EC_{50} values of vinyl sulfone **19** against its saturated counterpart **27** (0.66 μM versus 26.40 μM) the influence of the electron-poor vinyl group is clear. Comparison between **19** and its corresponding ammonium salt **25** (0.66 μM versus 22.40 μM) appears to indicate that the more polar **25** is not taken up by the cells as efficiently since the substrate profiling studies, discussed above, clearly demonstrate that positively charged residues in S1 are happily accommodated by this type of parasitic protease. Structural alteration in the S1' pocket across compounds **19**, **20** and **21** seems to lead to only modest alteration of activity (see also Figure 5, A). Contrasting Michael-type acceptors, **19** versus **22**, does not dramatically alter EC_{50}

values (0.66 μM versus 0.82 μM). However, α,β-unsaturated ketone **22** is significantly more active in the HL-60 cell line (Figure 2). This latter effect may be attributed to its enhanced electrophilicity, versus the corresponding sulfone members from the compound panel. Probing the S2 pocket, EC_{50} values for leucine **23** and more dramatically, proline **24**, are higher than phenylalanine **19** (see also Figure 5, C). Contrasting the group appended from the lysine nitrogen proved worthwhile: Sulfonamides **28**, **30** and particularly **29** all killed *T.b.b.* cells with lower EC_{50} values than carbamate (Boc) **19** which may be explained by more pronounced affinity within the S1 binding region. The activities of our synthetic compounds were compared with the benchmark vinyl sulfone, K777 (**2**).



Compounds: **19**-**25**, **27**-**30**



K777 (**2**)

Compound	R	R'		R''	EWG	*T.b.b.* EC_{50} (μM)	Selectivity *vs* HL-60
19	H	Bn	(Phe)	Boc	SO_2Ph	0.66	>83
20	H	Bn	(Phe)	Boc	SO_2(2-Naphthyl)	1.03	>53.4
21	H	Bn	(Phe)	Boc	SO_2(1-Naphthyl)	0.68	>80.1
22	H	Bn	(Phe)	Boc	COPh	0.82	>67.1
23	H	*i*-Bu	(Leu)	Boc	SO_2Ph	1.18	>46.6
24	-$(CH_2)_3$-		(Pro)	Boc	SO_2Ph	9.96	>5.7
25	H	Bn	(Phe)	H·HCl	SO_2Ph	22.40	>2.4
27[a]	H	Bn	(Phe)	Boc	SO_2Ph	26.40	>2.1
28	H	Bn	(Phe)	Ts	SO_2Ph	0.25	>220.0
29	H	Bn	(Phe)	SO_2(1-Naphthyl)	SO_2Ph	0.07	>785.7
30	H	Bn	(Phe)	SO_2[2-BrC_6H_2(4,5-OMe)$_2$]	SO_2Ph	0.31	>177.4
K777 (**2**)		-				5.56	>9.9

[a]6,7-Saturated (see Scheme 4)

Table 1. EC_{50} values and selectivity indices for inhibitors **19**-**25**, **27**-**30** and **2**.

Based on the trends summarized in Table 1, compound **29,** when directly compared with K777 (**2**), they both exhibited similar effects in terms of morphological changes to the parasite assessed using

epifluorescence microscopy. In the untreated sample the asynchronous population comprised parasites containing one kinetoplast and one nucleus (1K1N), two kinetoplasts and one nucleus (2K1N) and two kinetoplasts with two nuclei respectively (2K2N). However, treatment with either K777 (**2**) or **29** appeared to cause a failure in cytokinesis as indicated by the dramatic increase in the number of multinucleate cells observed as depicted in Panels A-D. In addition, many cells exhibited a morphology similar to the "bigeye" phenotype observed upon knockdown of the clathrin heavy chain.[37] This morphology follows the loss of cell polarity and most likely emerges from a block in endocytic recycling.

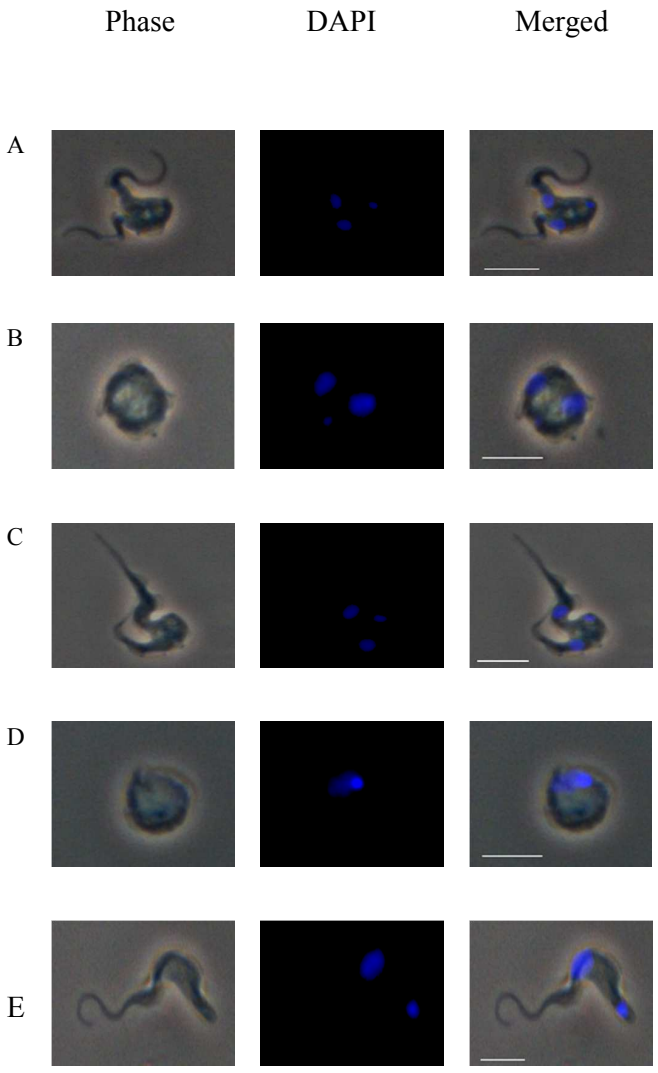


Figure 3. All cells were visualized using a Zeiss Axiovert 100 fluorescence microscope. The images were captured and processed using Axiovision software. Bar = 5 μm; Panel A-E. The DAPI column presents the fluorescence image with the nucleus/kinetoplast (blue). The merged column presents the corresponding merge with the phase contrast view. Panels A and B and C and D depict the predominant two morphological changes observed in *T.b.brucei* post-treatment with K777 (**2**) and compound **29** respectively. Panel E shows an untreated control.

Computational

The genomes of *Trypanosoma spp*. encode two Clan CA C1 family cysteine proteases, a cathepsin L-like enzyme brucipain (a.k.a. rhodesain/trypanopain-Tb) and a cathepsin B-like enzyme termed TbCatB, the only proteases produced by the parasite during mammalian-host infection. In 2009, the first crystal structure of rhodesain bound to a vinyl sulfone inhibitor, *N*-methyl-piperazine-Phe-hPhe-phenyl vinyl sulfone **2**, was released providing additional understanding of the substrate-binding site profile.[20] The vinyl sulfone inhibitor spans the S1'-S3 subsites and forms a Michael-type adduct with the active site cysteine thiol, Cys25. The ability of each subsite in accommodating a range of substituents has been assessed by numerous groups (see also above) and has been recently reviewed by Nicoll-Griffith.[38] In particular, *T.b.brucei* proteases were subsequently crystallized by Kerr *et al*.[39] enabling delineation of the key interactions between enzyme and inhibitor and rational design of newer higher affinity inhibitors by several other groups.[40-44] Yang *et al*. reported a strong correlation between the potency of rhodesain inhibition and whole-cell trypanocidal activity.[45] As a result, we have undertaken a computational study utilizing X-ray structures of TbCatB and rhodesain available in the protein data bank (www.wwpdb.org) to correlate whole-cell activity for a series of our compounds against *T.b.brucei* with 'on-target' binding mode to both TbCatB and rhodesain and in agreement with Yang *et al.*[45], a clear structure-activity relationship (SAR) emerges.

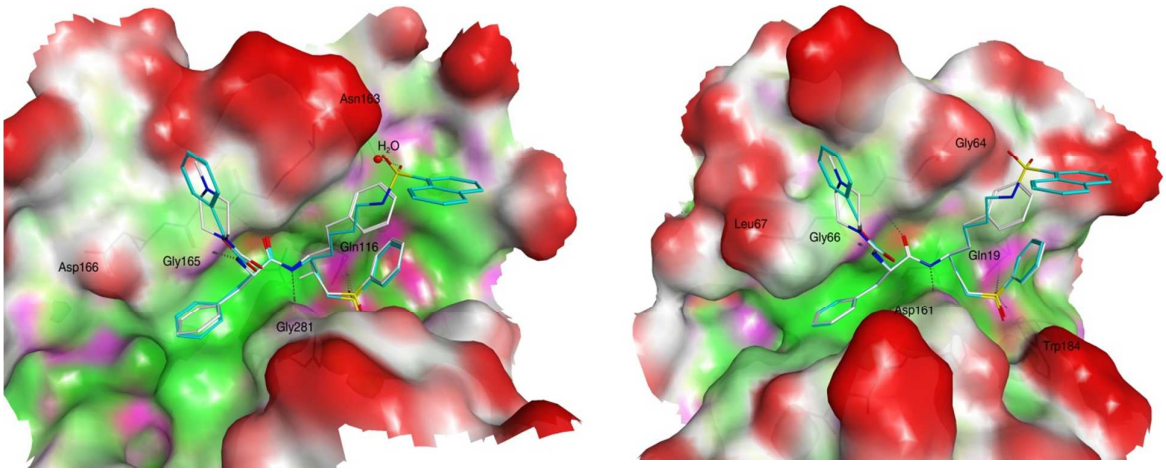


Figure 4. Binding pose of compound **29** in TbCatB (left) compared with Rhodesain (right) with compound K777 (**2**) also depicted (white).

As shown in Figure 4, compound **29** exhibits the same key hydrogen-bonding interactions with both TbCatB and rhodesain as observed with K777 (**2**), locking the peptide backbone in position *via* Gly281 (Asp161). Likewise, the vinyl sulfone oxygen atoms form crucial hydrogen bonds with Trp304 (Trp184) and Gln116 (Gln19). A clear difference between compound **29** binding to rhodesain compared with TbCatB is observed, whereby, Asn163 is capable of forming a hydrogen bond with the naphthylsulfonylamido moiety *via* a water-mediated interaction. In rhodesain, this is not possible since Asn163 is mutated to a glycine residue (Gly64) which bears no side-chain for interaction. Compounds **28-30** all bound in this manner suggesting one possible explanation for their nM trypanocidal activity compared to other members within the series.

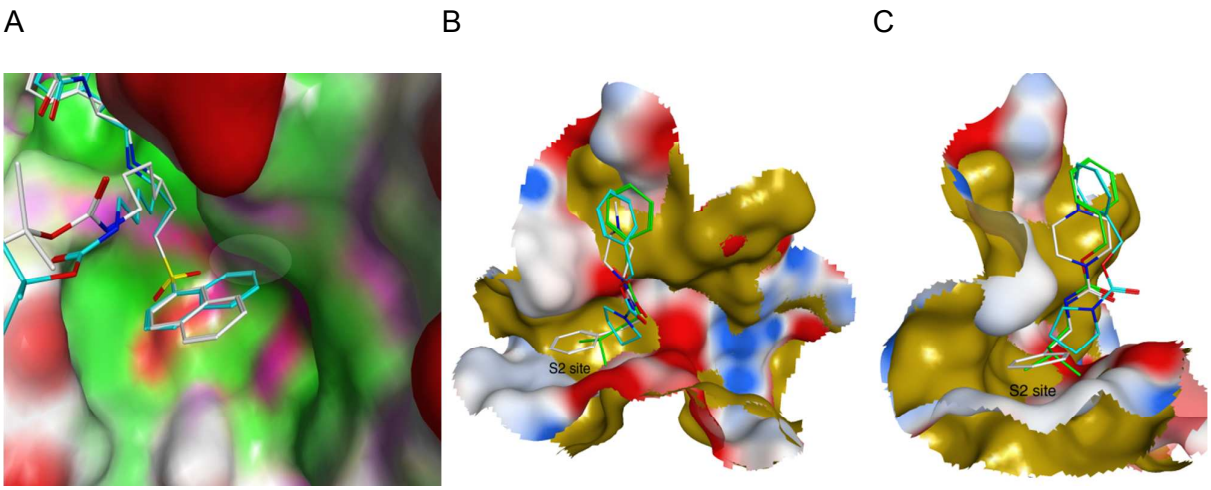


Figure 5. Binding poses of: **A**; compounds **20** (white) and **21** (cyan) in rhodesain (colored by electrostatics), **B** and **C**; compounds **19** (white), **23** (green) and **24** (cyan) in TbCatB (gold surface) and rhodesain (colored by electrostatics).

The 2-vinylsulfonylnapthalene of **20** versus 1-vinylsulfonylnapthalene of **21** causes a modest reduction in trypanocidal activity due to its less than ideal positioning against the wall formed by Cys25 (Cys122) as highlighted in Figure 5A. Changing from a phenylalanine sidechain in **19** to leucine, **23**, or proline, **24**, causes a significant loss in trypanocidal activity and can be rationalized by the depth to which the S2 site is penetrated by the different side-chains at P2. Figure 5B depicts proline barely occupying the deep hydrophobic S2 pocket and we hypothesize that this would result in a significant loss in affinity and subsequent loss in trypanocidal activity. The S2 pocket has a clear preference for bulky non-polar residues at P2, also noted by Brömme *et al*. in Cathepsin L,[15] which correlates well with the observed increase in trypanocidal potencies on replacement of P2 with Pro<Leu<Phe.

Conclusion

In summary, a series of lysine-containing dipeptide vinyl sulfones were synthesized and their trypanocidal effects investigated. Optimum potency was observed with phenylalanine, as opposed

to leucine or proline, as the additional amino acid fragment. Whilst the free amine **25**, itself was not particularly active, more lipophilic, non-basic *N*-derivatives such as *tert*-butyloxycarbamate **19** and sulfonamides **28** to **30** demonstrated potent killing of *Trypanosoma brucei brucei in vitro*. In addition, low cytotoxicity levels were observed in HL-60 cells (typically 2 orders of magnitude higher than the EC_{50} values recorded). The importance of the thiophilic vinyl sulfone unit was demonstrated, since upon hydrogenation the resultant saturated sulfone, **27**, proved significantly less active than its electrophilic precursor **19**. Finally, a novel computational study was performed which was successful in correlating binding preference for both TbcatB and rhodesain with whole-cell trypanocidal activity.

5. Experimental

General

Reagents were obtained from commercial suppliers and were used without further purification. Dry THF was distilled from sodium benzophenone ketyl radical and dry DCM distilled from calcium hydride, under nitrogen. Thin-layer chromatography was performed on silica coated aluminium sheets (60 F_{254}) supplied by Merck. Compounds were visualised with UV light and basic aqueous potassium permanganate, followed by heating. Flash column chromatography was performed using flash silica 60 Å (230-400 mesh) 9385 supplied by Merck. ^{1}H and ^{13}C NMR spectra were recorded using Varian Inova 300 MHz, 400 MHz and 500 MHz instruments, as indicated. Deuterochloroform and d_6-dimethyl sulfoxide were used as the solvents and chemical shifts are given in parts per million (ppm) relative to the standard reference TMS or residual protonated solvent. Peak assignment was achieved using 2-dimensional, ^{1}H-^{1}H and ^{1}H-^{13}C NMR experiments. Infrared spectra were recorded on a Varian Instruments Excalibur series FT-IR 3100 spectrometer. Samples were prepared neat between NaCl discs or deposited as films using dichloromethane.

Melting points were recorded on a Gallenkamp electrothermal melting point apparatus. High resolution mass spectra were carried out on a VG analytical 70-E mass spectrometer. Optical rotation data was obtained using a Perkin Elmer Model 343 polarimeter, with the sample solvent and values are quoted in units of 10^{-1}degcm^{2}g^{-1}. The purity of compounds **19-25** and **27-30** was determined by HPLC. The analysis was performed on an LC-2010A Shimadzu HPLC and an Agilent microsorb-MV 100-5 C-18 250 x 4.6 mm column was used for all samples. For compounds **19-24** and **27-30**, the column was eluted with an isocratic mixture of 60% acetonitrile, 30% water and 10% 0.1 M ammonium bicarbonate solution in water. For the ammonium salt **25**, the column was eluted with an isocratic mixture of 50% acetonitrile, 40% water and 10% of a 2% TFA solution in water. Detection was at 220 and 254 nm and the average peak area was used to determine purity: **19**, 97.0%; **20**, 98.0%; **21**, 98.8%; **22**, 91.6%; **23**, 95.5%; **24**, 90.8%; **25**, 99.0%; **27**, 93.2%; **28**, 94.1%; **29**, 96.3%; **30**, 97.8%.

Methyl 2*S*-(2*S*-benzyloxycarbonylamino-3-phenylpropanamido)-6-(*tert*-butoxycarbonylamino)hexanoate 9.

Under nitrogen, DIPEA (1.28 mL, 7.4 mmol, 1.1 equiv.) was added to a stirred solution of **5** (2.0 g, 6.7 mmol, 1 equiv.), **8** (2.0 g, 6.7 mmol, 1 equiv.) and EDCI·HCl (1.28 g, 6.7 mmol, 1 equiv.) in dichloromethane (65 mL). This mixture was left to stir at room temperature overnight under a balloon of nitrogen. 1 M HCl (20 mL) was added along with dichloromethane (20 mL) and the solution washed with water (20 mL). After drying the organic layer with $MgSO_4$ and filtering, the solvent was removed *in vacuo* to yield a white solid **9** (3.27 g, 90%); m.p. 98-104°C [lit. m.p. 121°C].[46] R_f = 0.4 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3416, 3326, 3056, 2932, 1709, 1511, 739 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.16-1.28 (m, 2H, CH_2), 1.40-1.48 (m, 11H, *t*-Bu, CH_2), 1.56-1.84 (m, 2H, CH_2), 3.01-3.14 (m, 4H, CH_2), 3.69 (s, 3H, CH_3), 4.40-4.56 (m, 2H, CH), 4.66 (s (br), 1H, NH), 5.09 (s, 2H, CH_2), 5.40 (s (br), 1H, NH), 6.35 (s (br), 1H, NH), 7.16-7.35 (m, 10H, ArH)

ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.2 (CH_2), 28.7 (CH_3), 29.5 (CH_2), 32.0 (CH_2), 38.5 (CH_2), 40.2 (CH_2), 52.2 (CH), 52.6 (CH_3), 56.5 (CH), 67.2 (CH_2), 79.0 (C), 127.1 (CH), 128.1 (CH), 128.2 (CH), 128.5 (CH), 128.7 (CH), 129.3 (CH), 136.1 (C), 136.2 (C), 155.9 (CO), 156.1 (CO), 170.7 (CO), 172.1 (CO) ppm. HRMS (ES^+): $C_{29}H_{40}N_3O_7$ calcd. 542.2866; found 542.2852. $[\alpha]_D$ = -9.8 (*c* = 0.1, $CHCl_3$), $[\alpha]_D$ = -7.2 (*c* = 1.0, MeOH), [lit. $[\alpha]_D$ = -9.6 (*c* = 1.0, MeOH)].[46]

2*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-6-(*tert*-butoxycarbonylamino)hexanol 12.

Under nitrogen, **9** (1.6 g, 3.0 mmol, 1 equiv.) was stirred with LiCl (1.0 g, 23.6 mmol, 8 equiv.) in THF (12 mL) and EtOH (6 mL). Once all solids had dissolved $NaBH_4$ (0.9 g, 23.8 mmol, 8 equiv.) was added and the solution left to stir overnight at room temperature. The solution was cooled to 0°C and 1 M HCl was added until the pH was ~2. The solution was diluted with dichloromethane (20 mL), washed with water (20 mL), dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to yield **12** (1.39 g, 92%) as a white solid; m.p. 110-114°C. R_f = 0.1 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3321, 3064, 2930, 2861, 1693, 1531, 1455, 748 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.15-1.56 (m, 15H, *t*-Bu, CH_2), 2.06 (s (br), 1H, OH), 2.97-3.18 (m, 4H, CH_2), 3.35-3.43 (m, 2H, CH_2), 3.77-3.84 (m, 1H, CH), 4.35-4.43 (m, 1H, CH), 4.75 (s (br), 1H, NH), 5.06 (app. t, *J* = 12.5 Hz, 2H, CH_2), 5.65 (s (br), 1H, NH), 6.13 (d, *J* = 7.5 Hz, 1H, NH), 7.16-7.36 (m, 10H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.8 (CH_2), 28.5 (CH_3), 29.6 (CH_2), 30.1 (CH_2), 38.7 (CH_2), 39.8 (CH_2), 51.4 (CH), 56.6 (CH), 64.1 (CH_2), 67.0 (CH_2), 79.2 (C), 127.0 (CH), 128.0 (CH), 128.1 (CH), 128.5 (CH), 128.6 (CH), 129.3 (CH), 136.1 (C), 136.6 (C), 156.1 (CO), 156.3 (CO), 171.1 (CO) ppm. HRMS (ES^+): $C_{28}H_{40}N_3O_6$ calcd. 514.2917; found 514.2905. $[\alpha]_D$ = -18.1 (*c* = 0.35, $CHCl_3$).

2*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-6-(*tert*-butoxycarbonylamino)hexanal.

At room temperature PCC (0.26 g; 1.2 mmol, 3 equiv.) was stirred with Celite (0.26 g) in dichloromethane (20 mL). Thereafter, **12** (0.2 g; 0.4 mmol, 1 equiv.), dissolved in dichloromethane (5 mL), was added. This was left to stir at room temperature for 6 hours. Excess solvent was removed *in vacuo* and the mixture taken-up in diethyl ether (10 mL) and filtered through silica washing with ethyl acetate (50 mL). The solvent was removed *in vacuo* to yield the aldehyde (0.11 g, 55%) as a creamy coloured solid; m.p. 110-124°C. R_f = 0.2 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3424, 2924, 2851, 1729, 1686, 1648, 1527 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.10-1.85 (m, 15H, *t*-Bu, CH_2), 2.90-3.10 (m, 4H, CH_2), 4.28-4.44 (m, 2H, CH), 4.57 (s (br), 1H, NH), 5.03 (s (br), 2H, CH_2), 5.32 (s (br), 1H, NH), 6.34 (d, *J* = 6.0 Hz, 1H, NH), 7.10-7.30 (m, 10H, ArH), 9.32 (s, 1H, CHO) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.2 (CH_2), 28.3 (CH_2), 28.6 (CH_3), 29.9 (CH_2), 38.6 (CH_2), 40.1 (CH_2), 56.4 (CH), 58.7 (CH), 67.3 (CH_2), 79.3 (C), 127.3 (CH), 128.2 (CH), 128.4 (CH), 128.7 (CH), 128.9 (CH), 129.5 (CH), 136.4 (C), 136.7 (C), 156.2 (CO), 156.3 (CO), 171.6 (CO), 198.9 (CO) ppm. HRMS (ES^+): $C_{28}H_{38}N_3O_6$ calcd. 512.2761; found 512.2770. $[\alpha]_D$ = -10.3 (*c* = 0.1, $CHCl_3$).

***trans*-5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(phenylsulfonyl)hept-6-en-1-*tert*-butyloxycarbonylamine 19.**

Under nitrogen, 60% w/w NaH in mineral oil (13 mg, 0.35 mmol, 1.2 equiv.) was stirred in THF (3 mL). At 0°C diethyl phenylsulfonylmethylphosphonate **15** (111 mg, 0.38 mmol, 1.3 equiv.), dissolved in THF (2 mL), was added and the mixture was left to stir for 30 minutes. Then the above aldehyde (149 mg, 0.29 mmol, 1 equiv.), dissolved in THF (6 mL), was added to the solution which was left to stir overnight during which time room temperature was gradually reached. Water (20 mL) was added to dilute the solution and it was washed with dichloromethane (20 mL) and brine (20 mL). After drying with $MgSO_4$ and filtering, the solvent was removed *in vacuo*. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 1:1) yielded **19** (98 mg, 52%) as a white solid;

m.p. 130-136°C. R_f = 0.3 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3375, 3068, 2928, 1663, 1529, 1366, 1147, 1084 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ = 1.12-1.66 (m, 15H, *t*-Bu, CH_2), 2.94-3.13 (m, 4H, CH_2), 4.33 (app. q, *J* = 7.0 Hz, 1H, CH), 4.55-4.70 (m, 2H, NH, CH), 5.08 (app. t, *J* = 14.0 Hz, 2H, CH_2), 5.37 (s (br), 1H, NH), 5.88 (d, *J* = 8.0 Hz, 1H, NH), 6.05 (d, *J* = 15.0 Hz, 1H, CH), 6.75 (dd, *J* = 5.0, 15.0 Hz, 1H, CH), 7.10-7.40 (m, 10H, ArH), 7.50-7.68 (m, 3H, ArH), 7.85 (d, *J* = 7.5 Hz, 2H, ArH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 22.5 (CH_2), 28.4 (CH_3), 29.3 (CH_2), 33.5 (CH_2), 38.2 (CH_2), 40.0 (CH_2), 49.2 (CH), 56.6 (CH), 67.2 (CH_2), 79.0 (C), 127.3 (CH), 127.6 (CH), 128.0 (CH), 128.3 (CH), 128.5 (CH), 128.8 (CH), 129.2 (CH), 129.3 (CH), 130.4 (CH), 133.5 (CH), 136.0 (C), 136.1 (C), 140.1 (C), 145.4 (CH), 156.0 (CO), 156.1 (CO), 170.8 (CO) ppm. HRMS (ES^+): $C_{35}H_{44}N_3O_7S$ calcd. 650.2900; found 650.2930. $[\alpha]_D$ = -11.5 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18) MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 26.79 min.

trans-5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(2''-naphthylsulfonyl)hept-6-en-1-*tert*-butyloxycarbonylamine 20.

PCC (291 mg, 1.40 mmol, 3 equiv.) was stirred with Celite (291 mg) in dichloromethane (15 mL). Alcohol **12** (229 mg; 0.50 mmol, 1 equiv.) was dissolved in dichloromethane (10 mL) and added to the PCC solution. This was left to stir at room temperature for 6 hours. Excess solvent was removed *in vacuo* and the mixture redissolved in diethyl ether (15 mL). It was filtered through silica and washed with ethyl acetate (50 mL). The solvent was removed *in vacuo* to yield the crude dipeptide aldehyde. Under nitrogen, 60% w/w NaH in mineral oil (22 mg, 0.55 mmol, 1.2 equiv.) was stirred in THF (2 mL). At 0°C, diethyl (naphthalen-2-ylsulfonyl)methylphosphonate **16** (200 mg, 0.60 mmol, 1.3 equiv.), dissolved in THF (3 mL), was added to the NaH mixture and left to stir for 30 minutes. The crude dipeptide aldehyde, dissolved in THF (5 mL), was added to the solution and it was left to stir overnight warming gradually to room temperature. Water (15 mL) was added to dilute the solution and it was washed with dichloromethane (3 x 15 mL) and brine (15 mL).

After drying with $MgSO_4$ and filtering, the solvent was removed *in vacuo*. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 9:1 to 1:1) yielded **20** (173 mg, 55%) as a white amorphous solid; m.p. 121-125°C. R_f = 0.4 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3309, 3056, 2932, 2840, 1701, 1523, 1452, 1310, 1253, 1162, 1035, 739 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 1.15-1.65 (m, 15H, *t*-Bu, CH_2), 2.95-3.10 (m, 4H, CH_2), 4.33 (app. q, *J* = 7.5 Hz, 1H, CH), 4.55-4.70 (m, 2H, CH, NH), 5.00-5.10 (m, 2H, CH_2), 5.41 (s (br), 1H, NH), 6.00 (s (br), 1H, NH), 6.14 (d, *J* = 15.5 Hz, 1H, CH), 6.80 (dd, *J* = 4.5, 15.5 Hz, 1H, CH), 7.08-7.36 (m, 10H, ArH), 7.60-7.69 (m, 2H, ArH), 7.76 (d, *J* = 8.5 Hz, 1H, ArH), 7.91 (d, *J* = 8.0 Hz, 1H, ArH), 7.98 (t, *J* = 8.0 Hz, 2H, ArH), 8.45 (s, 1H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.5 (CH_2), 28.4 (CH_3), 29.4 (CH_2), 33.5 (CH_2), 40.0 (CH_2), 40.1 (CH_2), 49.3 (CH), 56.6 (4-CH), 67.2 (CH_2), 79.2 (C), 122.5 (CH), 127.2 (CH), 127.7 (CH), 128.0 (CH), 128.1 (CH), 128.3 (CH), 128.6 (CH), 128.8 (CH), 129.20 (CH), 129.25 (CH), 129.3 (CH), 129.4 (CH), 129.6 (CH), 130.6 (CH), 132.3 (C), 135.2 (C), 136.00 (C), 136.05 (C), 137.0 (C), 145.4 (CH), 156.0 (CO), 156.1 (CO), 170.7 (CO) ppm. HRMS (ES^+): $C_{39}H_{45}N_3O_7SNa$ calcd. 722.2876; found 722.2859. $[\alpha]_D$ = -5.8 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 41.24 min.

trans-5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(1''-naphthylsulfonyl)hept-6-en-1-*tert*-butyloxycarbonylamine 21.

PCC (259 mg, 1.2 mmol, 3 equiv.) was stirred with Celite (259 mg) in dichloromethane (5 mL). Alcohol **12** (208 mg; 0.4 mmol, 1 equiv.) was dissolved in dichloromethane (10 mL) and added to the PCC solution. This was left to stir at room temperature for 6 hours. Excess solvent was removed *in vacuo* and the mixture redissolved in diethyl ether (15 mL). It was filtered through silica and washed with ethyl acetate (50 mL). The solvent was removed *in vacuo* to yield the crude dipeptide aldehyde. Under nitrogen, 60 % w/w NaH in mineral oil (19 mg, 0.5 mmol, 1.2 equiv.) was stirred in THF (2 mL). At 0°C, diethyl (naphthalen-1-ylsulfonyl)methylphosphonate **17** (180

mg, 0.5 mmol, 1.3 equiv.), dissolved in THF (3 mL), was added to the NaH mixture and left to stir for 30 minutes. The crude dipeptide aldehyde, dissolved in THF (5 mL), was added to the solution and it was left to stir overnight warming gradually to room temperature. Water (15 mL) was added to dilute the solution and it was washed with dichloromethane (3 x 15 mL) and brine (15 mL). After drying with $MgSO_4$ and filtering, the solvent was removed *in vacuo*. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 9:1 to 1:1) yielded **21** (114 mg, 41%) as a colourless oil which gradually solidified; m.p. 88-90°C. R_f = 0.3 (*c*-Hex: EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3309, 3056, 2932, 2840, 1701, 1523, 1452, 1310, 1253, 1162, 1035, 739 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ = 1.10-1.60 (m, 15H, *t*-Bu, CH_2), 2.90-3.08 (m, 4H, CH_2), 4.29 (app. q, *J* = 7.5 Hz, 1H, CH), 4.56-4.69 (m, 2H, CH, NH), 5.00-5.08 (m, 2H, CH_2), 5.40 (s (br), 1H, NH), 6.00 (s (br), 1H, NH), 6.23 (d, *J* = 15.0 Hz, 1H, CH), 6.86 (dd, *J* = 5.0, 15.0 Hz, 1H, CH), 7.05-7.35 (m, 10H, ArH), 7.57-7.67 (m, 3H, ArH), 7.95 (d, *J* = 8.0 Hz, 1H, ArH), 8.12 (d, *J* = 8.0 Hz, 1H, ArH), 8.32 (d, *J* = 8.0 Hz, 1H, ArH), 8.53 (d, *J* = 8.0 Hz, 1H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.5 (CH_2), 28.4 (CH_3), 29.3 (CH_2), 33.6 (CH_2), 38.2 (CH_2), 40.1 (CH_2), 49.3 (CH), 56.6 (CH), 67.2 (CH_2), 79.1 (C), 124.2 (CH), 124.5 (CH), 127.0 (CH), 127.2 (CH), 128.1 (CH), 128.3 (CH), 128.5 (CH), 128.6 (CH), 128.8 (CH), 129.16 (CH), 129.19 (CH), 129.9 (CH), 130.9 (CH), 134.2 (C), 134.9 (C), 135.3 (CH), 136.00 (C), 136.05 (C), 136.1 (C), 145.4 (CH), 156.0 (CO), 156.1 (CO), 170.7 (CO) ppm. HRMS (ES^+): $C_{39}H_{45}N_3O_7SNa$ calcd. 722.2876; found 722.2858. $[\alpha]_D$ = -14.0 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 39.29 min.

trans-4*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-8-(*tert*-butyloxycarbonylamino)oct-2-en-1-one 22.

PCC (556 mg, 2.6 mmol, 3 equiv.) was stirred with Celite (556 mg) in dichloromethane (15 mL). Alcohol **12** (442 mg, 0.9 mmol, 1 equiv.) was dissolved in dichloromethane (15 mL) and added to

the PCC solution. This was left to stir at room temperature for 6 hours. Excess solvent was removed *in vacuo* and the mixture redissolved in diethyl ether (15 mL). It was filtered through silica and washed with ethyl acetate (60 mL). The solvent was removed *in vacuo* to yield the crude dipeptide aldehyde. Under nitrogen, 60% w/w NaH in mineral oil (47 mg, 1.2 mmol, 1.3 equiv.) was stirred in THF (2 mL). At 0°C diethyl 2-oxo-2-phenylethylphosphonate **18** (300 mg, 1.2 mmol, 1.3 equiv.), dissolved in THF (3 mL), was added to the NaH mixture and left to stir for 30 minutes. The crude dipeptide aldehyde, dissolved in THF (5 mL), was added to the solution and it was left to stir overnight warming gradually to room temperature. Water (10 mL) was added to dilute the solution and it was washed with dichloromethane (20 mL) and brine (50 mL). After drying with $MgSO_4$ and filtering, the solvent was removed *in vacuo*. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 2:1 to 1:1) yielded **22** (74 mg, 13%) as a yellow, amorphous solid; m.p. 85-87°C. R_f = 0.4 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3440, 2929, 2861, 1690, 1625, 1527, 1454, 1042 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.20-1.85 (m, 15H, *t*-Bu, CH_2), 2.90-3.20 (m, 4H, CH_2), 4.43 (app. q, *J* = 7.0 Hz, 1H, CH), 4.60-4.81 (m, 2H, CH, NH), 5.00-5.10 (m, 2H, CH_2), 5.35-5.55 (m, 1H, NH), 6.08 (s (br), 1H, NH), 6.68 (dd, *J* = 5.5, 15.5 Hz, 1H, CH), 6.80 (d, *J* = 15.5 Hz, 1H, CH), 7.10-7.35 (m, 10H, ArH), 7.47 (t, *J* = 8.0 Hz, 2H, ArH), 7.57 (t, *J* = 7.5 Hz, 1H, ArH), 7.91 (d, *J* = 7.5 Hz, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.7 (CH_2), 28.4 (CH_3), 29.5 (CH_2), 33.8 (CH_2), 38.3 (CH_2), 40.1 (CH_2), 50.3 (CH), 56.6 (CH), 67.2 (CH_2), 79.2 (C), 125.2 (CH), 127.2 (CH), 127.3 (CH), 128.0 (CH), 128.2 (CH), 128.5 (CH), 128.6 (CH), 128.7 (CH), 128.8 (CH), 133.0 (CH), 135.6 (C), 136.0 (C), 137.5 (C), 146.7 (CH), 156.05 (CO), 156.10 (CO), 170.6 (CO), 190.2 (CO) ppm. HRMS (ES^+): $C_{36}H_{44}N_3O_6$ calcd. 636.3050; found 636.3031. $[\alpha]_D$ = -6.7 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 43.28 min.

Methyl 2*S*-(2*S*-benzyloxycarbonylamino-4-methylpentanamido)-6-(*tert*-butoxycarbonylamino)hexanoate 10.

Under nitrogen, DIPEA (1.75 mL, 10.0 mmol, 1.1 equiv.) was added to a stirred solution of **6** (2.4 g, 9.1 mmol, 1 equiv.), **8** (2.7 g, 9.1 mmol, 1 equiv.) and EDCI·HCl (1.8 g, 9.4 mmol, 1.05 equiv.) in dichloromethane (90 mL). This mixture was left to stir at room temperature overnight under a balloon of nitrogen. 1 M HCl (10 mL) was added along with dichloromethane (20 mL) and the solution washed with water (20 mL). After drying the organic layer with $MgSO_4$ and filtering, the solvent was removed *in vacuo* to yield **10** (4.2 g, 91%) as a white solid; m.p. 68-71°C [lit. m.p. 84.5-85°C].[47] R_f = 0.4 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3339, 2955, 2931, 2870, 1708, 1665, 1528, 1519, 1438, 1454 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.90-0.98 (m, 6H, CH_3), 1.20-1.90 (m, 18H, *t*-Bu, CH_2, CH), 3.00-3.10 (m, 2H, CH_2), 3.74 (s (br), 3H, CH_3), 4.15-4.25 (m, 1H, CH), 4.54-4.61 (m, 1H, CH), 4.71-4.78 (m, 1H, NH), 5.12 (s (br), 2H, CH_2), 5.27-5.34 (m, 1H, NH), 6.48-6.56 (m, 1H, NH), 7.28-7.38 (m, 5H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.0 (CH_3), 22.2 (CH_3), 22.9 (CH_2), 24.6 (CH), 28.4 (CH_3), 29.1 (CH_2), 31.9 (CH_2), 40.1 (CH_2), 41.2 (CH_2), 51.8 (CH), 52.4 (CH_3), 53.5 (CH), 67.1 (CH_2), 79.2 (C), 128.0 (CH), 128.2 (CH), 128.5 (CH), 136.2 (C), 156.0 (CO), 156.2 (CO), 172.0 (CO), 172.5 (CO) ppm. HRMS (ES^+): $C_{26}H_{42}N_3O_7$ calcd. 508.3023; found 508.3007. $[\alpha]_D$ = -12.6 (*c* = 0.1, $CHCl_3$) [lit. $[\alpha]_D$ = -5.6 (*c* = 1.0, EtOAc)].[47]

2*S*-(2*S*-Benzyloxycarbonylamino-4-methylpentanamido)-6-(*tert*-butoxycarbonylamino)hexanol 13.

Under nitrogen, **10** (3.9 g, 7.7 mmol, 1 equiv.) was stirred with LiCl (2.6 g, 61.5 mmol, 8 equiv.) in THF (52 mL) and EtOH (26 mL). Once all solids had dissolved $NaBH_4$ (3.3 g, 61.5 mmol, 8 equiv.) was added and the solution left to stir overnight at room temperature. Product formation was confirmed by TLC. The solution was cooled to 0°C and 1 M HCl (40 mL) was added until the pH was ~2. The solution was diluted with dichloromethane (30 mL), washed with water (30 mL), dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to yield **13** (3.6 g, 97%) as a colourless foam; m.p. 75-83°C. R_f = 0.1 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3331, 2956, 2934,

2870, 1694, 1659, 1531, 1455 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.88-0.97 (m, 6H, CH_3), 1.24-1.78 (m, 18 H, *t*-Bu, CH_2, CH), 2.85-3.20 (m, 3H, CH_2, OH), 3.52-3.65 (m, 2H, CH_2), 3.84-3.94 (m, 1H, CH), 4.12-4.20 (m, 1H, CH), 4.73 (s (br), 1H, NH), 5.11 (s (br), 2H, CH_2), 5.37-5.44 (m, 1H, NH), 6.35-6.41 (m, 1H, NH), 7.29-7.38 (m, 5H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.0 (CH_3), 22.6 (CH_2), 22.9 (CH_3), 24.7 (CH), 28.4 (CH_3), 29.6 (CH_2), 30.0 (CH_2), 39.7 (CH_2), 41.3 (CH_2), 51.4 (CH), 53.8 (CH), 64.5 (CH_2), 67.1 (CH_2), 79.3 (C), 128.1 (CH), 128.2 (CH), 128.5 (CH), 136.1 (C), 156.3 (CO), 156.4 (CO), 172.6 (CO) ppm. HRMS (ES^+): $C_{25}H_{41}N_3O_6Na$ calcd. 502.2893; found 502.2905. $[\alpha]_D$ = -35.6 (*c* = 0.1, $CHCl_3$).

trans-5*S*-(2'*S*-Benzyloxycarbonylamino-4'-methylpentanamido)-7-(phenylsulfonyl)hept-6-en-1-*tert*-butoxycarbonylamine 23.

PCC (0.4 g, 1.8 mmol, 3 equiv.) was stirred with Celite (0.4 g) in dichloromethane (20 mL). A solution of **13** (0.3 g; 0.6 mmol, 1 equiv.) dissolved in dichloromethane (~10 mL) was added. This was left to stir at room temperature for 6 hours. Excess solvent was removed *in vacuo* and the mixture taken-up in diethyl ether (10 mL). It was filtered through silica and washed with ethyl acetate (50 mL). The solvent was removed *in vacuo* to yield the crude dipeptide aldehyde. Under nitrogen, 60% w/w NaH in mineral oil (28 mg, 0.7 mmol, 1.2 equiv.) was stirred in THF (3 mL). At 0°C diethyl phenylsulfonylmethylphosphonate **15** (239 mg, 0.8 mmol, 1.3 equiv.), dissolved in THF (2 mL), was added to the NaH mixture and left to stir for 30 minutes. The crude dipeptide aldehyde, dissolved in THF (6 mL), was added to the solution and it was left to stir overnight warming gradually to room temperature. Water (40 mL) was added to dilute the solution and it was washed with dichloromethane (30 mL) and brine (70 mL). After drying with $MgSO_4$ and filtering, the solvent was removed *in vacuo*. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 2:1 to 1:1) yielded **23** (168 mg, 46%) as a colourless oil which gradually solidified; m.p. 88-92°C. R_f = 0.5 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3337, 3068, 2955, 2918, 1656, 1630, 1447,

1366, 1148, 778 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.82-0.96 (m, 6H, CH_3), 1.20-1.70 (m, 18H, *t*-Bu, CH_2, CH), 2.98-3.08 (m, 2H, CH_2), 4.12-4.19 (m, 1H, CH), 4.61-4.69 (m, 1H, CH), 4.75-4.82 (m, 1H, NH), 5.04-5.12 (m, 2H, CH_2), 5.44-5.50 (m, 1H, NH), 6.45 (d, *J* = 15.0 Hz, 1H, CH), 6.60-6.70 (m, 1H, NH), 6.87 (dd, *J* = 5.0, 15.0 Hz, 1H, CH), 7.26-7.37 (m, 5H, ArH), 7.48-7.63 (m, 3H, ArH), 7.82-7.88 (m, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 (CH_3), 22.6 (CH_2), 22.8 (CH_3), 24.6 (CH), 28.4 (CH_3), 29.2 (CH_2), 33.3 (CH_2), 40.1 (CH_2), 40.8 (CH_2), 49.1 (CH), 53.6 (CH), 67.2 (CH_2), 79.2 (C), 127.6 (CH), 128.0 (CH), 128.2 (CH), 128.5 (CH), 129.3 (CH), 130.6 (CH), 133.5 (CH), 136.0 (C), 140.1 (C), 145.7 (CH), 156.1 (CO), 156.5 (CO), 172.2 (CO) ppm. HRMS (ES^+): $C_{32}H_{46}N_3O_7S$ calcd. 616.3056; found 616.3085. $[\alpha]_D$ = -25.6 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 26.77 min.

Benzyl 2*S*-(6-*tert*-butoxycarbonylamino)-1-methoxy-1-oxohexan-2*S*-ylcarbamoyl)pyrrolidine-1-carboxylate 11.

Under nitrogen, DIPEA (0.8 mL, 4.6 mmol, 1.1 equiv.) was added to a stirred solution of **7** (1.0 g, 4.0 mmol, 1 equiv.), **8** (1.2 g, 4.0 mmol, 1 equiv.) and EDCI·HCl (0.7 g, 4.0 mmol, 1 equiv.) in dichloromethane (40 mL). This mixture was left to stir at room temperature overnight under a balloon of nitrogen. 1 M HCl (10 mL) was added along with dichloromethane (15 mL) and the solution washed with water (10 mL). After drying the organic layer with $MgSO_4$ and filtering, the solvent was removed *in vacuo* to yield **11** (1.8 g, 90%) as a white solid; m.p. 80-82°C, [lit. m.p. 92-93°C].[48] R_f = 0.5 (*c*-Hex-EtOAc; 1:5). IR (film): $\bar{\nu}$ = 3477, 3393, 2917, 1739, 1659, 1542, 1423, 876 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.20-1.50 (m, 13H, *t*-Bu, CH_2), 1.50-2.34 (m, 6H, CH_2), 3.00-3.10 (m, 2H, CH_2), 3.40-3.72 (m, 5H, CH_3, CH_2), 4.30-4.35 (m, 1H, CH), 4.49-4.57 (m, 1.4H, CH, NH), 4.80 (s (br), 0.6H, NH), 5.17 (s (br), 2H, CH_2), 6.47 (s (br), 0.35H, NH), 7.05 (s (br), 0.65H, NH), 7.28-7.39 (m, 5H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.3 (CH_2), 24.6 (CH_2), 28.4 (CH_3), 29.3 (CH_2), 31.85 (CH_2), 31.90 (CH_2), 40.1 (CH_2), 47.0 (CH_2), 52.0 (CH), 52.3

(CH_3), 60.4 (CH), 67.3 (CH_2), 78.9 (C), 127.8 (CH), 128.0 (CH), 128.5 (CH), 136.4 (C), 156.0 (2 x CO), 171.5 (CO), 172.6 (CO) ppm. HRMS (ES^+): $C_{25}H_{37}N_3O_7$ calcd. 492.2710; found 492.2686. $[\alpha]_D$ = -40.0 (*c* = 0.1, $CHCl_3$), [lit. $[\alpha]_D$ = -24.4 (*c* = 1.0, DMF)].[48] Note NMR spectra complicated due to mixtures of rotamers.

Benzyl 2*S*-(6-*tert*-butoxycarbonylamino)-1-hydroxyhexan-2*S*-ylcarbamoyl)pyrrolidine-1-carboxylate 14.

Under nitrogen, methyl ester **11** (1.5 g, 3.0 mmol, 1 equiv.) was stirred with LiCl (1.0 g, 23.6 mmol, 8 equiv.) in THF (20 mL) and EtOH (10 mL). Once all solids had dissolved $NaBH_4$ (0.9 g, 23.7 mmol, 8 equiv.) was added and the solution left to stir overnight at room temperature. Product formation was confirmed by TLC. The solution was cooled to 0°C and 1 M HCl (20 mL) was added until the pH was ~2. The solution was diluted with dichloromethane (20 mL), washed with water (20 mL), dried with $MgSO_4$, filtered and the solvent removed *in vacuo* to yield **14** (1.2 g, 87%) as a white powder; m.p. 85-87°C. R_f = 0.1 (*c*-Hex-EtOAc; 1:2). IR (film): $\bar{\nu}$ = 3695, 3342, 3099, 2932, 2876, 1690, 1530, 1421 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.21-1.63 (m, 15H, *t*-Bu, CH_2), 1.85-2.33 (m, 4H, CH_2), 2.82 (s (br), 0.6H, OH), 2.99-3.17 (m, 2H, CH_2), 3.37-3.69 (m, 4H, CH_2), 3.80-3.90 (m, 1H, CH), 4.27-4.34 (m, 1H, CH), 4.52-4.70 (m, 1H, NH), 5.03-5.26 (m, 2H, CH_2), 6.18 (s (br), 0.35H, NH), 6.59 (s (br), 0.65H, NH), 7.28-7.40 (m, 5H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.8 (CH_2), 24.6 (CH_2), 28.4 (CH_3), 28.8 (CH_2), 29.7 (CH_2), 30.2 (CH_2), 40.0 (CH_2), 47.1 (CH_2), 51.8 (CH), 61.0 (CH), 65.0 (CH_2), 67.4 (CH_2), 79.1 (C), 128.0 (CH), 128.2 (CH), 128.5 (CH), 136.3 (C), 156.2 (2 x CO), 172.3 (CO) ppm. HRMS (ES^+): $C_{24}H_{37}N_3O_6Na$ calcd. 486.2580; found 486.2570. $[\alpha]_D$ = -64.8 (*c* = 0.1, $CHCl_3$). Note NMR spectra complicated due to mixtures of rotamers.

***trans*-Benzyl 2*S*-[(1-*tert*-butoxycarbonylamino)-7-(phenylsulfonyl)hept-6-en-5*S*-ylcarbamoyl]pyrrolidine-1-carboxylate 24.**

PCC (0.7 g; 3.2 mmol, 3 equiv.) was stirred with Celite (0.7 g) in dichloromethane (20 mL). A solution of **14** (0.5 g; 1.1 mmol, 1 equiv.) in dichloromethane (~10 mL) was added. This was left to stir at room temperature for 6 hours. Excess solvent was removed *in vacuo* and the mixture redissolved in diethyl ether (10 mL). It was filtered through silica and washed with ethyl acetate (50 mL). The solvent was removed *in vacuo* to yield a creamy coloured solid. Under nitrogen, 60% w/w NaH in mineral oil (52 mg, 1.3 mmol, 1.2 equiv.) was stirred in THF (3 mL). At 0°C diethyl phenylsulfonylmethylphosphonate **15** (409 mg, 1.4 mmol, 1.3 equiv.), dissolved in THF (2 mL), was added to the NaH mixture and left to stir for 30 minutes. The crude dipeptide aldehyde, dissolved in THF (6 mL), was added to the solution and it was left to stir overnight warming gradually to room temperature. Water (30 mL) was added to dilute the solution and it was washed with dichloromethane (20 mL) and brine (20 mL). After drying with $MgSO_4$ and filtering, the solvent was removed *in vacuo*. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 2:1) yielded **24** (313 mg, 53%) as a colourless oil which gradually solidified; m.p. 98-101°C. R_f = 0.1 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3419, 3371, 2918, 2850, 1695, 1638, 1447, 1420, 1363, 1147, 924 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.20-2.35 (m, 19H, *t*-Bu, CH_2), 2.95-3.15 (m, 2H, CH_2), 3.38-3.58 (m, 2H, CH_2), 4.25-4.35 (m, 1H, CH), 4.50-4.70 (m, 2H, CH, NH), 5.00-5.25 (m, 2H, CH_2), 6.20-6.57 (m, 2H, CH), 6.78-6.93 (m, 2H, CH, NH), 7.25-7.65 (m, 8H, ArH), 7.75-7.95 (m, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.8 (CH_2), 24.7 (CH_2), 28.4 (CH_3), 29.4 (CH_2), 30.0 (CH_2), 33.6 (CH_2), 40.2 (CH_2), 47.1 (CH_2), 49.2 (CH), 60.5 (CH), 67.5 (CH_2), 79.1 (C), 127.6 (CH), 127.8 (CH), 128.0 (CH), 128.4 (CH), 129.3 (CH), 130.5 (CH), 133.4 (CH), 136.2 (C), 140.1 (C), 145.8 (CH), 156.0 (CO), 156.3 (CO), 171.3 (CO) ppm. HRMS (ES^+): $C_{31}H_{42}N_3O_7S$ calcd. 600.2743; found 600.2738. $[\alpha]_D$ = -39.0 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 21.54 min.

***trans*-5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(phenylsulfonyl)hept-6-en-1-ammonium chloride 25.**

A solution of **19** (108 mg, 0.16 mmol, 1 equiv.) in a 70% mixture of TFA (0.7 mL) in acetic acid (0.3 mL) was stirred at room temperature for one hour. Excess solvent was removed *in vacuo* before ethyl acetate (~15 mL) was added. This mixture was extracted with water (5 x 10 mL) and the combined aqueous layers were then concentrated *in vacuo* which gave the trifluoroacetate salt. This salt was redissolved in water (15 mL) and treated for 48 hr with IRA400 Amberlite resin in its chloride form (1 g).[34] The resin was removed by filtration, washing with water (5 mL), and evaporation of the water afforded the ammonium chloride salt **25** (84 mg, 89%) as a white solid; m.p. 74°C (decomp.). R_f = 0.0 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3416, 2919, 1642, 1426, 1150, 1080 cm^{-1}. ^1H NMR (400 MHz, d_6-DMSO): δ = 1.21-1.65 (m, 6H, CH_2), 2.65-2.81 (m, 3H, CH_2), 2.88-2.96 (m, 1H, CH_2), 4.17-4.25 (m, 1H, CH), 4.43-4.52 (m, 1H, CH), 4.92 (d, *J* = 12.5 Hz, 1H, CH_2), 4.97 (d, *J* = 12.5 Hz, 1H, CH_2), 6.42 (d, *J* = 15.0 Hz, 1H, CH), 6.82 (dd, *J* = 5.0, 15.0 Hz, 1H, CH), 7.13-7.38 (m, 9H, ArH), 7.52 (d, *J* = 8.5 Hz, 1H, NH), 7.62-7.68 (m, 2H, ArH), 7.76-7.70 (m, 2H, ArH), 7.79-7.93 (m, 4H, NH_3, ArH), 8.23 (d, *J* = 8.0 Hz, 1H, NH) ppm. ^{13}C NMR (100 MHz, d_6-DMSO): δ = 22.1 (CH_2), 26.4 (CH_2), 32.3 (CH_2), 37.4 (CH_2), 38.4 (CH_2), 48.9 (CH), 56.4 (CH), 65.3 (CH_2), 126.8 (CH), 127.5 (CH), 127.9 (CH), 128.2 (CH), 128.5 (CH), 128.7 (CH), 129.6 (CH), 129.9 (CH), 130.0 (CH), 134.1 (CH), 136.9 (C), 137.6 (C), 140.3 (C), 147.2 (CH), 155.8 (CO), 171.2 (CO) ppm. HRMS (ES^+): $C_{30}H_{36}N_3O_5S$ calcd. 550.2376; found 550.2366. $[\alpha]_D$ = -21.9 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-2% v/v $TFA_{(aq)}$; 50:40:10 (0.4 mL/min): t_r = 16.19 min.

5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(phenylsulfonyl)heptan-1-*tert*-butyloxycarbonylamine 27.

Dipeptide vinyl sulfone **19** (110 mg, 0.17 mmol, 1 equiv.) was sonicated in ethanol (10 mL) until the substrate was completely dissolved and 10% Pd/C (18 mg, 0.017 mmol 0.1 equiv.) was added. This mixture was stirred in an atmosphere of hydrogen (1 atm.) for a total of 26 hours. The reaction

mixture was filtered through a pad of Celite which was subsequently washed with ethyl acetate (3 x 15 mL). Solvent was removed *in vacuo* to give the crude product which was purified by column chromatography (*c*-Hex-EtOAc 2:1 to 1:1) to give reduced product **27** (47 mg, 42%) as a white powdery solid; m.p. 139-144°C. R_f = 0.2 (*c*-Hex-EtOAc; 1:1). IR (film): $\bar{\nu}$ = 3360, 3312, 3059, 2934, 2862, 1685, 1653, 1531, 1448, 1368, 1266, 1151, 738 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ = 1.05-1.90 (m, 17H, *t*-Bu, CH_2), 2.75-3.10 (m, 6H, CH_2), 3.80-3.90 (m, 1H, CH), 4.29 (app. q, *J* = 7.0 Hz, 1H, CH), 4.60 (s (br), 1H, NH), 5.08 (app. t, *J* = 13.0 Hz, 1H, CH_2), 5.33 (s (br), 1H, NH), 5.66 (d, *J* = 8.5 Hz, 1H, NH), 7.10-7.38 (m, 10H, ArH), 7.57 (t, *J* = 8.0 Hz, 2H, ArH), 7.67 (t, *J* = 8.0 Hz, 1H, ArH), 7.87 (d, *J* = 8.0 Hz, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.7 (CH_2), 27.9 (CH_2), 28.5 (CH_3), 29.7 (CH_2), 34.7 (CH_2), 37.9 (CH_2), 40.2 (CH_2), 48.0 (CH), 53.0 (CH_2), 56.7 (CH), 67.3 (CH_2), 79.2 (C), 127.3 (CH), 128.0 (CH), 128.2 (CH), 128.3 (CH), 128.6 (CH), 128.9 (CH), 129.2 (CH), 129.3 (CH), 133.8 (CH), 136.0 (C), 136.3 (C), 139.2 (C), 156.00 (CO), 156.05 (CO), 171.0 (CO) ppm. HRMS (ES^+): $C_{35}H_{45}N_3O_7SNa$ calcd. 674.2876; found 674.2883. $[\alpha]_D$ = -5.0 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 23.87 min.

trans-5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(phenylsulfonyl)hept-6-en-1-(4-methylphenyl)sulfonamide 28.

Ammonium chloride salt **25** (20 mg, 0.03 mmol, 1 equiv.) was stirred with 4-toluene sulfonyl chloride (10 mg, 0.05 mmol, 1.5 equiv.) in DCM (2 mL) at 0°C. Triethylamine (13 μL, 0.10 mmol, 3 equiv.) was added as a solution in DCM (1 mL) and the reaction was stirred overnight warming slowly to room temperature. Water (3 mL) was added followed by DCM (3 mL). The aqueous layer was re-extracted twice with DCM (2 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and solvent was removed *in vacuo* to yield the crude product. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 2:1) yielded sulfonamide **28** (14 mg, 58%) as a

white solid; m.p. 144-146 °C. R_f = 0.7 (*c*-Hex-EtOAc; 1:2). IR (film): $\bar{\nu}$ = 3305, 3036, 2926, 2856, 1706, 1665, 1532, 1319, 1147 cm^{-1}. ^1H NMR (400 MHz, d_6-DMSO): δ = 1.07-1.53 (m, 6H, CH_2), 2.35 (s, 3H, CH_3), 2.60-2.68 (m, 2H, CH_2), 2.69-2.78 (m, 1H, CH), 2.84-2.93 (m, 1H, CH), 4.15-4.22 (m, 1H, CH), 4.37-4.46 (m, 1H, CH), 4.86-4.97 (m, 2H, CH_2), 6.37 (d, *J* = 15.5 Hz, 1H, CH), 6.78 (dd, *J* = 15.5 Hz, 5.0 Hz, 1H, CH), 7.13-7.39 (m, 12H, ArH), 7.45 (t, *J* = 6.0 Hz, 1H, NH), 7.50 (d, *J* = 8.0 Hz, 1H, NH), 7.61-7.68 (m, 4H, ArH), 7.69-7.75 (m, 1H, ArH), 7.82 (d, *J* = 7.0 Hz, 2H, ArH), 8.09 (d, *J* = 7.0 Hz, 1H, NH) ppm. ^{13}C NMR (100 MHz, d_6-DMSO): δ = 20.9 (CH_3), 22.3 (CH_2), 28.6 (CH_2), 32.5 (CH_2), 37.4 (CH_2), 42.3 (CH_2), 48.9 (CH), 56.2 (CH), 65.2 (CH_2), 126.3 (CH), 126.5 (CH), 127.1 (CH), 127.5 (CH), 127.7 (CH), 128.0 (CH), 129.1 (CH), 129.3 (CH), 129.6 (2 × CH), 133.6 (CH), 136.9 (C), 137.6 (2 × C), 140.3 (C), 142.5 (C), 147.2 (C), 155.7 (CO), 171.1 (CO) ppm. HRMS (ES^+): $C_{37}H_{41}N_3O_7S_2Na$ calcd. 726.2284; found 726.2308. $[\alpha]_D$ = -20.0 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 32.12 min.

trans-5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(phenylsulfonyl)hept-6-en-1-(1-naphthalene)sulfonamide 29.

Ammonium chloride salt **25** (21 mg, 0.036 mmol, 1 equiv.) was stirred with 1-naphthalene sulfonyl chloride (12 mg, 0.053 mmol, 1.5 equiv.) in DCM (2 mL) at 0°C. To this was added triethylamine (12 μL, 0.086 mmol, 3 equiv.) as a solution in DCM (1 mL). The reaction mixture was allowed to stir overnight warming slowly to room temperature. Water (3 mL) was added followed by DCM (3 mL). The aqueous layer was re-extracted twice with DCM (2 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and solvent was removed *in vacuo* to yield the crude product. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 2:1) yielded naphthalene sulfonamide **29** (18 mg, 67%) as a white solid; m.p. 74-78 °C. R_f = 0.7 (*c*-Hex-EtOAc; 1:2). IR (film): $\bar{\nu}$ = 3350, 3033, 2955, 2925, 2854, 1706, 1666, 1539, 1318, 1146 cm^{-1}. ^1H NMR

(400 MHz, $CDCl_3$): δ = 1.11-1.50 (m, 6H, CH_2), 2.72-2.93 (m, 2H, CH_2), 3.04 (d, *J* = 7.5 Hz, 2H, CH_2), 4.39 (app. q, *J* = 7.5 Hz, 1H, CH), 4.54 (s (br), 1H, CH), 5.04 (s, 2H, CH_2), 5.38 (s, 1H, $NHSO_2$), 5.63 (s, 1H, NH), 5.97 (d, *J* = 15.5 Hz, 1H, CH), 6.02 (d, *J* = 8.5 Hz, 1H, NH), 6.69 (dd, *J* = 15.5, 4.5 Hz, 1H, CH), 7.10-7.22 (m, 5H, ArH), 7.24-7.34 (m, 5H, ArH), 7.48-7.59 (m, 4H, ArH), 7.60-7.67 (m, 2H, ArH), 7.84 (d, *J* = 7.5 Hz, 2H, ArH), 7.92 (d, *J* = 8.0 Hz, 1H, ArH), 8.05 (d, *J* = 8.0 Hz, 1H, ArH), 8.21 (d, *J* = 8.0 Hz, 1H, ArH), 8.68 (d, *J* = 8.5 Hz, 1H, ArH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.6 (CH_2), 28.2 (CH_2), 32.9 (CH_2), 38.2 (CH_2), 42.3 (CH_2), 49.0 (CH), 56.8 (CH), 67.2 (CH_2), 124.2 (CH), 124.5 (CH), 126.9 (CH), 127.2 (CH), 127.7 (CH), 127.9 (CH), 128.12 (C), 128.16 (CH), 128.2 (CH), 128.5 (CH), 128.8 (CH), 129.15 (CH), 129.23 (CH), 129.3 (CH), 129.5 (CH), 130.2 (CH), 133.5 (CH), 134.2 (CH), 134.3 (C), 134.9 (C), 136.0 (C), 136.1 (C), 140.1 (C), 145.5 (CH), 156.3 (CO), 171.2 (CO) ppm. HRMS (ES^+): $C_{40}H_{41}N_3O_7S_2Na$ calcd. 762.2284; found 762.2316. $[\alpha]_D$ = -15.0 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 31.19 min.

***trans*-5*S*-(2'*S*-Benzyloxycarbonylamino-3'phenylpropanamido)-7-(phenylsulfonyl)hept-6-en-1-(2-bromo-4,5-dimethoxybenzene)sulfonamide 30.**

Ammonium chloride salt **25** (30 mg, 0.051 mmol, 1 equiv.) was stirred with 2-bromo-4,5-dimethoxybenzene sulfonyl chloride[49] (24 mg, 0.076 mmol, 1.5 equiv.) in DCM (2 mL) at 0°C. To this was added triethylamine (12 μL, 0.086 mmol, 3 equiv.) as a solution in DCM (1 mL). The reaction mixture was allowed to stir overnight warming slowly to room temperature. Water (3 mL) was added followed by DCM (5 mL). The aqueous layer was re-extracted twice with DCM (2 x 5 mL). The combined organic layers were dried over $MgSO_4$, filtered, and solvent was removed *in vacuo* to yield the crude product. Purification by silica gel column chromatography (*c*-Hex-EtOAc; 1:2) yielded sulfonamide **30** (23 mg, 55%) as a white solid; m.p. 69-74 °C. R_f = 0.6 (*c*-Hex-EtOAc 1:2). IR (film): υ = 3314, 3061, 2927, 2855, 1708, 1664, 1585, 1321, 1148 cm^{-1}. ^1H NMR (400

MHz, $CDCl_3$): δ = 1.21-1.71 (m, 6H, CH_2), 2.76-2.91 (m, 2H, CH_2), 3.05 (d, *J* = 7.0 Hz, 2H, CH_2), 3.89 (s, 3H, CH_3), 3.92 (s, 3H, CH_3), 4.35 (app. q, *J* = 7.0 Hz, 1H, CH), 4.63 (s, 1H, CH), 5.07 (s, 2H, CH_2), 5.27 (s, 1H, NH), 5.49 (s, 1H, NH), 6.02 (d, *J* = 8.0 Hz, 1H, NH), 6.11 (dd, *J* = 15.0, 1.5 Hz, 1H, CH), 6.76 (dd, *J* = 15.0, 4.5 Hz, 1H, CH), 7.12-7.24 (m, 6H, ArH), 7.27-7.36 (m, 5H, ArH), 7.51-7.59 (m, 3H, ArH), 7.60-7.67 (m, 1H, ArH), 7.85 (d, *J* = 7.5 Hz, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 (CH_2), 28.3 (CH_2), 33.0 (CH_2), 38.1 (CH_2), 42.3 (CH_2), 49.1 (CH), 56.4 (CH_3), 56.5 (CH_3), 56.6 (CH), 67.2 (CH_2), 111.0 (C), 113.9 (CH), 117.1 (CH), 127.2 (CH), 127.6 (CH), 128.0 (CH), 128.2 (CH), 128.5 (CH), 128.8 (CH), 129.2 (CH), 129.3 (CH), 130.3 (CH), 130.4 (CH), 133.5 (C), 136.0 (C), 136.2 (C), 140.0 (C), 145.5 (CH), 148.1 (C), 152.4 (C), 156.2 (CO), 171.1 (CO) ppm. HRMS (ES^+): $C_{38}H_{42}N_3O_9S_2{}^{79}BrNa$ calcd. 850.1444; found 850.1479. $[\alpha]_D$ = -9.0 (*c* = 0.1, $CHCl_3$). HPLC Analysis (C-18), MeCN-H_2O-0.1 M $NH_4HCO_{3(aq)}$; 60:30:10 (0.4 mL/min): t_r = 23.54 min.

Cell Culture

The bloodstream form *T. brucei brucei* MITat1.1 strain was used for assessment of compound sensitivity *in vitro*. *T. b. brucei* was cultured in antibiotic-free HMI-9 medium[50] supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biosera, UK). Trypanosomes were subcultured at the appropriate dilutions (1:10) every 24 hours in fresh HMI-9 medium to ensure log growth phase. HL-60, a human acute myeloblastic leukemia cell line, was kindly provided by Prof. Luke O'Neill (School of Biochemistry and Immunology, TBSI, TCD, Ireland) and was maintained in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biosera, UK), 100 units/ml penicillin and 0.1 mg/ml streptomycin, and 2 mM L-glutamine. All cultures were maintained in a humidified atmosphere containing 5% CO_2 at 37°C (*T. b. brucei* and HL-60 cells).

Viability Assays

The effect of our cysteine protease inhibitors on parasite growth and HL-60 cells was determined using the Alamar Blue assay, essentially as described by Räz *et al*.[51] Briefly, *T. brucei brucei* cells (strain MITat1.1) and HL-60 cells were seeded in 96-well plates at a density of 2×10^5 cells/ml in 100 µl media in the presence of varying concentrations of predicted inhibitors or DMSO alone. A further 30 µl of media was added to each well. After 6-8 h for *T.b.brucei* and 30-32 h for HL-60 cells, 15 µl Alamar Blue (Invitrogen) was added to the cells and incubation continued so that the total incubation time was 24 h, or 48 h respectively. Absorbances at 540 and 595 nm were measured using SpectraMax M3 Microplate Reader (Molecular Devices), and EC_{50} values were calculated using the GraphPad Prism 5 software.

Fluorescence microscopy

T.b.brucei were treated with **29** (0.78 µM) and K777 (55.6 µM) and incubated at 37°C for 24 h. Cells were fixed for 10 min at 37°C with 3% (w/v) paraformaldehyde final concentration. Following fixation, cells were washed once in PBS buffer containing sodium azide (15 mM) and once in water, before allowing the cells to settle on poly-L-lysine coated coverslips. The coverslips were mounted onto slides with Prolong gold to visualize the nucleus and kinetoplast and finally sealed.

Computational

The compound series were converted from 2D to 3D with CORINA v3.2 (Molecular Networks, GmbH) following prediction of protonation states *via* the pK_a predictor plugin, Marvinview (Chemaxon Ltd, http://www.chemaxon.com). X-ray structures of *T.brucei* Cathepsin B in complex with CA074 (3HHI) and Rhodesain in complex with inhibitor K777 (2P7U) were downloaded from the Protein Data Bank (http://www.rcsb.org). MOE v2011.10 (CCG, http://www.chemcomp.com)

was used to structurally align 3HHI to 2P7U and the transformed co-ordinates were saved. Retaining K777 as a template ligand, Torchv10 (Cresset Group, http://www.cresset-group.com) was used for the ligand-based alignment of all compounds, constraining conserved field-points. After re-insertion of the covalent adduct with the sulfur of the active site cysteine thiol for all ligands, optimization of each ligand in the active site of 3HHI and 2P7U using LigX (Chemaxon Ltd, http://www.chemaxon.com) was carried out to refine the structural interactions.

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Supporting Information Available: Experimental details concerning the synthesis of phosphonates **15-18**, scanned NMR spectra and HPLC traces. This material is available free of charge *via* the internet at http://pubs.acs.org.

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