Organic & Biomolecular Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/obc

UCD School of Chemistry and Chemical Biology
Scoil na Ceimice agus na Ceimbhitheolaiochta UCD

UCD Science Centre (South)
University College Dublin
Belfield, Dublin 4, Ireland

Ionad Léann agus (Theas) UCD
An Colaiste Ollschoile, Baile Atha Cliath
Belfield, Baile Atha Cliath 4, Éire

T +353 1 716 2291
F +353 1 716 1178

chemistry@ucd.ie
paul.evans@ucd.ie

6/8/2014

Re: OB-ART-07-2014-001412

Dear Sir or Madam,

Thank you for your recent correspondence, we are delighted that the referees were generally positive about our manuscript.

We have adjusted the manuscript and electronic supporting information in the light of the comments from Referee 1: Specifically: a sentence has been included at the end of the introduction to emphasise that the PCC method was in fact optimised during the work we reported in Ref. 3.

We're grateful to the referee for noticing an important typographical error in the numbering in Table 1 and have corrected this. I also noticed that we had omitted to include data for compound **21** (which was inactive) so have included it in this version.

We have considered the comments about the spectra and have looked again at this section. We've reprocessed the original FIDs (apodization along t1), expanded the spectra, so the peak patterns are clearer and occasionally labelled the minor peaks caused by traces of solvents (and water). In three cases we have included HPLC traces to further demonstrate purity (including the key dansyl derivative – compound **19**).

However, we don't really agree that the original spectra were any cause for concern in terms of levels of purity and would argue that for compounds not purified by preparative HPLC they are in fact rather clean! (excluding our previous work, no real ESI is available for compounds of this class). Apart from this, we would argue that they are representative of the material used in the biological assays, therefore researchers interested in these compounds and their activities are able to make judgements in terms of the biological results. I hope you think that this is reasonable and are satisfied with this re-worked section.

Finally, I noticed an issue with reference 1c and the missing authors have been added. We hope you are happy with these adjustments and thank you for your time.

Yours sincerely

Paul Evans

Preparation, anti-trypanosomal activity and localisation of a series of dipeptide-based vinyl sulfones

William Doherty,[a] Jinju James,[a] Paul Evans,[a*] Laura Martin,[b] Nikoletta Adler,[b] Derek Nolan,[b] and Andrew Knox[b]

[a]*Centre for Synthesis and Chemical Biology, School of Chemistry and Chemical Biology, University College Dublin, Dublin 4, Ireland. paul.evans@ucd.ie*

[b]*School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College Dublin, Pearse Street, Dublin 2, Ireland. aknox@tcd.ie*

Dedicated to Richard Taylor who first taught me about vinyl sulphones and Weinreb amides, on the occasion of his 65th birthday.

Abstract-An improved, Weinreb amide-based, synthesis of anti-trypanosomal lysine-containing vinyl sulfones is described incorporating, as a feature, diversity at the ε-lysine amino group. Members of this family demonstrated moderate to good efficacy as anti-trypanosomal agents and a fluorescent dansyl (**19**) derivative was used to investigate subcellular localisation of the compound class.

Introduction

Vinyl, or α,β-unsaturated, sulfones are recognised as useful synthetic intermediates and additionally have been incorporated into compounds possessing biological activity, or have found use as biological tools.[1] We have previously reported the synthesis and anti-trypanosomal[2] activities of a small library of peptidyl vinyl sulfones.[3] Biological evaluation of this library against *Trypanosoma brucei brucei*, led to identification of lysine-based compound **1** which showed good *in vitro* trypanosidal activity. The design of this series of compounds was inspired by the benchmark vinyl sulfone K777 (**2**) developed by McKerrow and colleagues as a potential treatment for Chagas' disease (American sleeping sickness).[4]

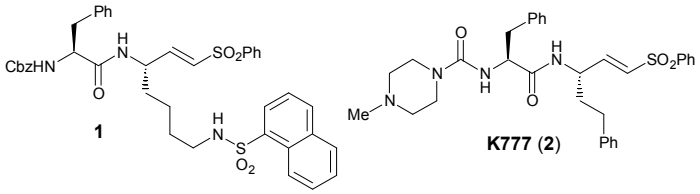
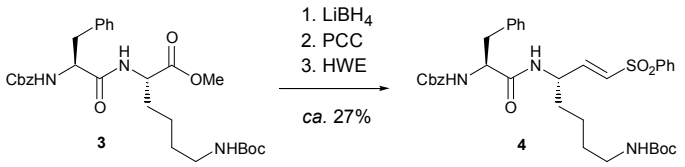


Figure 1. Lead compound **1** and K777 (**2**)

These compounds, at least in part,[4,5] mediate biological effects *via* disruption of lysosomal parasitic cysteinyl protease activity[6] and several X-ray crystal structures are available

indicating how the thiophilic vinyl sulfone entity forms a covalent bond with the catalytically active cysteine residue within the binding pocket of the protease.[7] The incorporation of the lysine group in compounds belonging to the series, including **1**, aimed to explore the well-documented substrate-cysteinyl protease complementarity.[8] Synthetically, vinyl sulfone **1** was prepared from vinyl sulfone **4** which in turn originated from a reduction/oxidation strategy performed on dipeptide methyl ester **3** (Scheme 1).[3]

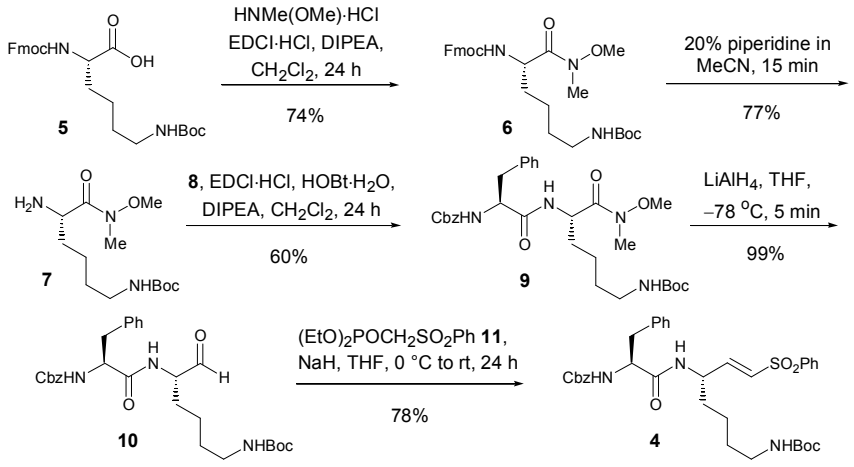


Scheme 1. Reduction/oxidation/HWE strategy

Although successful, the overall yield was modest and the biggest disadvantage of this synthetic sequence was a pyridinium chlorochromate (PCC) oxidation, which gave very poor yields of the sensitive, intermediate aldehyde - used subsequently in the Horner-Wadsworth-Emmons (HWE) olefination. It is worth noting that alternative methods of oxidation (Swern, Dess-Martin periodinane etc.) proved even less effective.[3] The current work describes an improved synthetic sequence to compounds of the type **1**, a series of new analogues designed to probe the putative target's P1 binding domain and their biological evaluation against *T. b. brucei.*

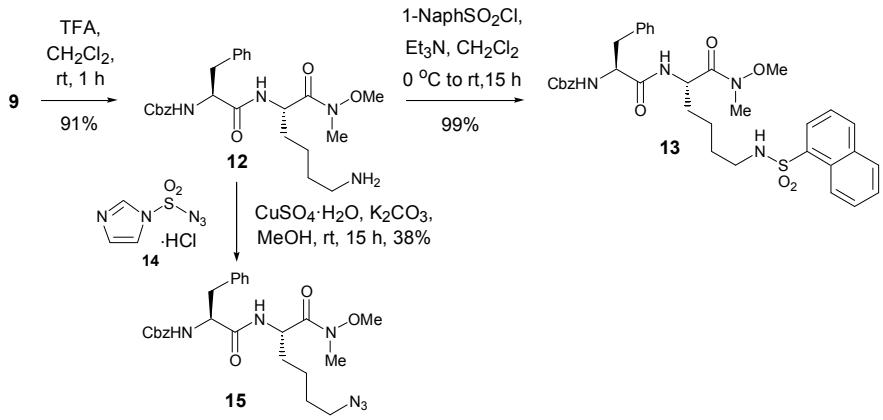
Chemistry

The issues observed with the described reduction-oxidation sequence led us to a newly devised route featuring use of a Weinreb amide[9] which provides an alternative means to access the dipeptide aldehyde (Scheme 2).[10] Starting with inexpensive differentially protected lysine **5**, EDCI coupling with *N,O*-dimethylhydroxylamine hydrochloride gave the Weinreb amide **6**, which was subsequently deprotected with piperidine in acetonitrile to give the free amine **7**. The amine could be used crude for the subsequent coupling with *N*-Cbz phenylalanine **8** to give the dipeptide Weinreb amide **9** in good yield. Treatment of **9** with lithium aluminium hydride at –78 °C in THF gave the desired dipeptide aldehyde **10** in excellent yield in stark contrast to the oxidation-based route outlined in Scheme 1. This material was submitted to a Horner-Wadsworth-Emmons olefination with the sodium salt of phosphonate **11** which gave the dipeptide vinyl sulfone **4** also in good yield as a single geometrical isomer.



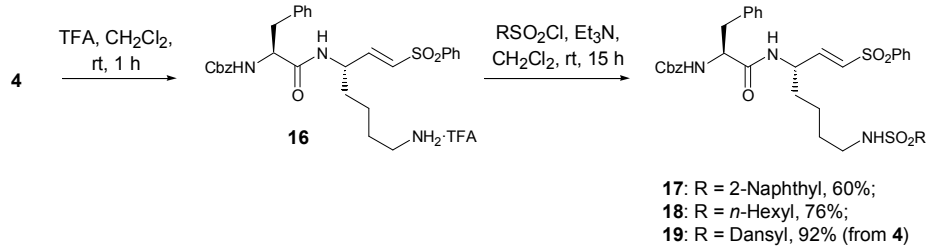
Scheme 2. Weinreb amide-based synthesis of dipeptide vinyl sulfone **4**

Prior to the elaboration of the lysine side chain of **4**, we also removed the *tert*-butyloxycarbonyl (Boc) protecting group from **9** and reacted the resulting free amine **12**, formed following basification of the trifluroacetate ammonium salt, with 1-naphthalenesulfonyl chloride to produce sulfonamide **13** (Scheme 3). Free amine **12** was also successfully converted into azide **15**, albeit in low yield, using the diazo-transfer reagent **14** reported by Goddard-Borger and co-workers.[11]



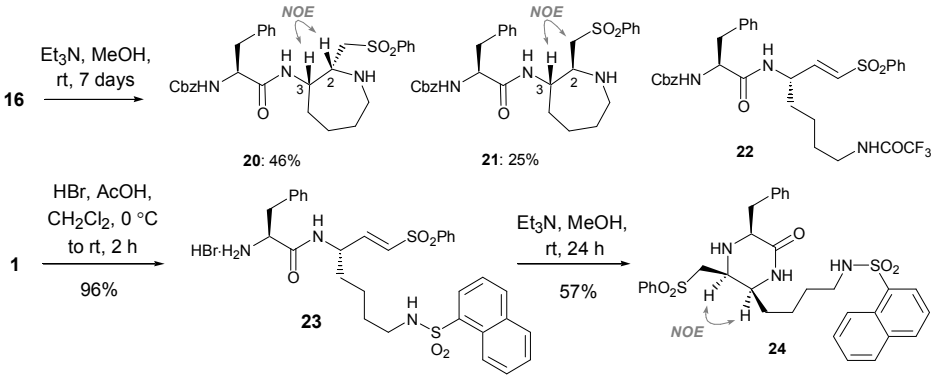
Scheme 3. Dipeptide Weinreb amide elaboration

Similarly, as shown in Scheme 4, in the presence of trifluroacetic acid vinyl sulfone **4** underwent removal of the Boc group generating ammonium salt **16** which was subsequently used without additional purification. The ammonium salt **16** was treated with two structurally different sulfonyl chlorides, which gave sulfonamides **17** (a regioisomer of our lead compound **1**) and aliphatic sulfonamide **18**. Additionally, the fluorescent dansyl group[12] was also incorporated into compound **19**.



Scheme 4. Sulfonamide formation from ammonium salt **16**

The successful derivatisation of **16** implies that intermolecular sulfonamide formation is rapid since the combination of a basic/nucleophilic primary amine and an electrophilic vinyl sulfone functional group might be expected to lead to an inter and/or intramolecular *N*-conjugate addition process.[13] This aspect was next examined and as shown in Scheme 5, basification of ammonium salt **16** in methanol,[13d] resulted in gradual formation of diastereomeric azepanes **20** and **21**, arising from intramolecular conjugate addition of the amine to the vinyl sulfone moiety.



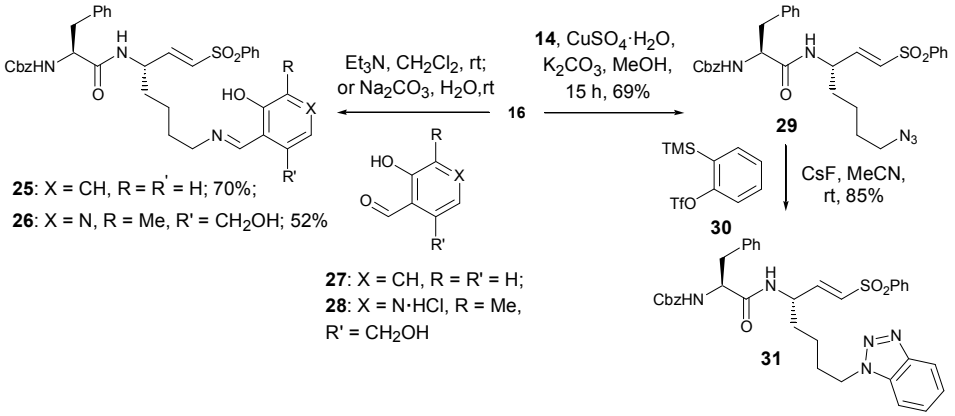
Scheme 5. Cyclisation studies

In addition, a small quantity of trifluoroacetamide **22** was also isolated - presumably formed during the reaction forming **16**. The relative stereochemistry of the chromatographically separable adducts **20** and **21** was confirmed by NOE measurements and it should be mentioned that no diastereoisomer interconversion was observed over time in deuterio-MeOH.[14] The dramatic differences in polarity and melting points between **20** and **21** are probably indicative of internal hydrogen bonding in adduct **20**. In a related study, the intramolecular conjugate addition of the dipeptide backbone was also considered. Thus, Cbz deprotection of **1** was achieved using HBr in acetic acid giving the corresponding ammonium

bromide salt **23**. Basification of this intermediate ammonium salt, under the Et_3N-MeOH conditions, gave adduct **24** as a single diastereoisomer.

Since this type of conjugate addition is, in principle at least, reversible we felt it was of interest to speculate whether β-amino sulfones, such as adducts **20**, **21** and **24**, might constitute masked vinyl sulfone cysteine protease inhibitors.

Based on the well-appreciated preference for parasitic cysteine proteases to digest proteins/polypeptides adjacent to a basic amino acid residue[8] it was initially felt that ammonium salts, such as **16**, might represent potent inhibitors. However, in the event, previous studies indicated that **16** was significantly less active than **1**.[3] A likely explanation for this discrepancy is that the ammonium salt does not efficiently accumulate in the trypanosomal cell. Consequently alternative methods were considered to improve lipophilicity whilst maintaining potential electrostatic inhibitor-enzyme interactions. Bearing this in mind a means to convert ammonium salt **16** into imines **25** and **26** using salicylaldehyde **27** and pyridoxal **28** respectively was developed (Scheme 6).



Scheme 6. Imine and Azide formation

Imine adducts of this type, possessing an internal hydrogen bond ("imine clip") have been synthesised previously in relation to bioorganic chemistry (amino acid decarboxylation/aminotransfer) and to provide a lipophilic vehicle for a primary amine.[15] It is envisaged that imine hydrolysis, facilitated by this intramolecular hydrogen bond, will result in the release the free amine/ammonium ion and either an innocuous, or a biologically active (eg. **28**) aldehyde.

Finally, diazo-transfer was also considered as an alternative means for mimicking, rather than temporarily masking, the amino side chain and ammonium salt **16** was converted into the more lipophilic azide **29** using the method described in Scheme 3.[11] The presence of the azido group also opens up possibilities for structural divergence using cycloaddition chemistry. In order to exemplify this point, following a literature method,[17] annulation of azide **29** with the benzyne precursor **30** gave benzotriazole **31** in good yield.

Biology

All vinyl sulfone members of the series were evaluated for their ability to disrupt the life cycle of *trypanosomes* in a whole-cell screen of *T. b. brucei via* a standard Alamar Blue assay.[17] As shown in Table 1, the benchmark lysine-containing vinyl sulfone **1** proved to be the most active compound with an EC_{50} of 0.74 μM (Entry 1). Ammonium salt **16**, presumably possessing poorer cell penetration, proved significantly less active despite presenting a cation to effectively interact with the well-described P1 binding domain[6,7] (Entry 2). Compound **13**, incorporating the same 1-naphthyl sulfonamide lysine derivative but with the Weinreb amide and not the vinyl sulfone functional group, proved inactive (Entry 3). Alternative ε-amino lysine derivatives, including the 2-naphthyl sulfonyl (**17**), *n*-hexyl sulfonyl (**18**), dansyl (**19**) and trifluoroacetyl (**22**), are all active, albeit slightly less so than compound **1** (Entries 4-7), a point demonstrating the flexibility associated with derivatives in this particular structural region of the likely protein target. Entries 8 and 9 indicate that the azepanes **20** and **21**, which might be considered to be masked vinyl sulfones, are not active in this assay. Similarly, imines **25** and **26** (and their aldehydes **27** and **28**) proved inactive at concentrations below 10 μM (Entries 10 and 11). Azide **29**, designed as a more lipophilic analogue of ammonium salt **16**, proved moderately active with an EC_{50} value of 4.93 μM (Entry 12). The importance of the vinyl sulfone was again demonstrated by the finding that Weinreb amide **15**, a direct analogue of azide **29**, was inactive (Entry 13). Finally, the benzotriazole **31**, with a similar topology in terms of the P1 region to the lysine sulfonamide **1**, proved to be active with an EC_{50} of 1.88 μM (Entry 14).

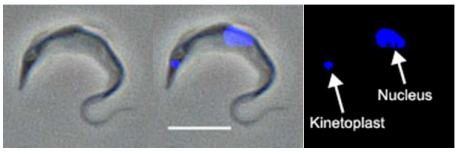
Entry	Compound	EC_{50}[a]	SE $logEC_{50}$[b]
1	**1**	0.74 μM	0.029
2	**16**	8.22 μM	0.213
3	**13**	>10 μM	ND
4	**17**	1.18 μM	0.028
5	**18**	1.22 μM	0.023
6	**19**	3.70 μM	0.092
7	**22**	3.30 μM	0.056
8	**20**	>10 μM	ND
9	**21**	>10 μM	ND
10[c]	**25**	>10 μM	ND
11[c]	**26**	>10 μM	ND
12	**29**	4.93 μM	0.045
13	**15**	>10 μM	ND
14	**31**	1.88 μM	0.084

[a]Response of *T. b. brucei* (2×10^5 cells/mL) to exposure of varying concentrations of each compound determined by non-linear regression analysis of curve-fitting using the equation Y = 100/[1+10^(logEC$_{50}$-X)*Hill slope]. [b]Standard errors (SE) were calculated based on the average of triplicate values from three independent experiments (ND = not determined). [c]EC_{50} values for aldehydes **26** and **27** >10 μM.

Table 1. Anti-trypanosomal activities of analogues

The reported fluorescence of the dansyl functional group[12] present in derivative **19** in conjunction with its inherent anti-trypanosomal properties (Table 1, Entry 6) led us to investigate whether it could serve to image *T. b. brucei* cells undergoing cell death as a direct response to this class of vinyl sulfone inhibitor.

(a)



(b)

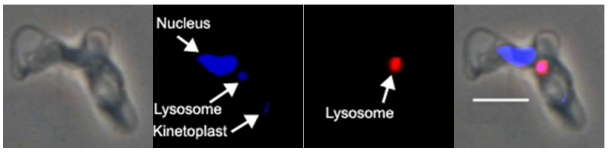


Figure 2. Representative images of *T. b. brucei* fixed after 24 h: (a) untreated cells stained with DAPI, (b) following treatment with derivative **19** and LysoTracker®. Images were

captured with Zeiss Axiovert 100 fluorescence microscope equipped with an AxioCam HRc camera. LysoTracker® Red DND-99 — Red, Derivative **19** – Blue. Scale bar = 5 μm.

The genomes of *Trypanosoma brucei brucei* encode two Clan CA C1 family cysteine proteases, a cathepsin L-like enzyme brucipain and a cathepsin B-like enzyme termed TbCatB.[6] Both brucipain and TbCatB have been previously shown to be localised in lysosomal vesicles and we set out to determine if our vinyl sulfone series, represented by derivative **19**, localised in a similar manner since we have postulated that they are most likely targeted to both cysteine proteases. Figure 2 clearly illustrates subcellular localisation of derivative **19** to the lysosome of *T. b. brucei* and its effects on parasite morphology were consistent with a defect in endocytic traffic, leading to an enlargement of the flagellar pocket ('big-eye' phenotype). Interestingly, localisation of derivative **19** also occurred in the nucleus and kinetoplast which may suggest additional, as yet unidentified target(s),[5] either as a result of binding of the dansyl moiety, or solely due to the vinyl sulfone scaffold.

Conclusions

In summary, a series of lysine-based vinyl sulfones were prepared using an efficient Weinreb amide strategy. These compounds, which differ at the ε-amino lysine position, were investigated for their ability to kill *T. b. brucei*. Optimum *N*-substituents are sulfonamides (compounds **1**, **17** and **18**) and inclusion of the dansyl group enabled subcellular localisation experiments to be carried out consistent with lysosomal targeting, most likely due to binding to trypanosomal cysteine proteases. Interestingly, derivative **19** also localised to the nucleus and kinetoplast of *T. b. brucei,* pointing to binding to additional proteins residing in those organelles. Additionally, the azide-benzyne "click" adduct **31** shows promising activity for future development.

General Experimental

^{1}H and ^{13}C NMR spectra were recorded on Varian Unity 600 MHz, 500 MHz and 400 MHz system spectrometers and coupling constants (*J*) are quoted in Hertz. High resolution mass spectra were carried out on a VG analytical 70-E mass spectrometer. Infrared spectra were recorded on a Varian Instruments Excalibur series FT-IR 3100 spectrometer. UV–Visible spectra were recorded in chloroform on a Varian Cary 50 UV–Vis spectrophotometer. Fluorescence spectra were recorded on a Varian Cary Eclipse spectrophotometer. Melting points were recorded on a Gallenkamp electrothermal melting point apparatus. Optical

rotation data was obtained with a Perkin Elmer Model 343 polarimeter and values are quoted in units of 10^{-1}degcm^{2}g^{-1}. Reagents were obtained from commercial suppliers and were used without further purification. Tetrahydrofuran was distilled from sodium-benzophenone ketyl radical. Thin-layer chromatography was performed on silica coated aluminium sheets and compounds were visualised with UV light and aqueous potassium permanganate, or ninhydrin, followed by heating.

(9*H*-Fluoren-9-yl)methyl *tert*-butyl [6-(methoxy(methyl)amino)-6-oxohexane-1,5*S*-diyl]dicarbamate 6.[18] Under nitrogen, **5** (3.88 g, 8.3 mmol, 1 equiv.) was stirred with DIPEA (1.45 mL, 8.3 mmol, 1 equiv.) in CH_2Cl_2 (80 mL). EDCI·HCl (1.00 g, 8.3 mmol, 1 equiv.) was added and the reaction mixture was stirred for 10 minutes before *N,O*–dimethylhydroxylamine hydrochloride (810 mg, 8.3 mmol, 1 equiv.) was added followed by a second portion of DIPEA (1.45 mL, 8.3 mmol, 1 equiv.). The reaction mixture was then left to stir overnight. The mixture was washed with 1 M HCl (2 × 20 mL), saturated sodium hydrogen carbonate (2 × 20 mL) and brine (2 × 20 mL). The organic layer was dried over $MgSO_4$, filtered and solvent was removed to give the crude product which was purified by column chromatography (*c*–Hex/EtOAc; 1:1) to give Weinreb amide **6** as a white foam (3.14 g, 74%). M.p. = 48–51 °C. R_f = 0.5 (*c*–Hex/EtOAc; 1:2). IR (film): ν_{max} 3320, 2975, 2934, 1709, 1654, 1522, 1451, 1391, 1366, 1249, 1171 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.34–1.67 (m, 13H, ^{t}Bu, CH_2) 1.71–1.82 (m, 2H, CH_2) 3.06–3.16 (m, 2H, CH_2) 3.22 (s, 3H, CH_3) 3.77 (s, 3H, CH_3) 4.22 (t, *J* = 7.0 Hz, 1H, CH) 4.37 (d, *J* = 7.0 Hz, 2H, CH_2) 4.58 (s (br), 1H, NH) 4.71–4.79 (m, 1H, CH) 5.57 (d, *J* = 9.0 Hz, 1H, NH) 7.28–7.34 (m, 2H, ArH) 7.39 (t, *J* = 7.0 Hz, 2H, ArH) 7.60 (t, *J* = 7.0 Hz, 2H, ArH) 7.76 (d, *J* = 7.0 Hz, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.6 (CH_2) 28.5 (CH_3) 29.7 (CH_2) 32.2 (CH_3) 32.6 (CH_2) 40.3 (CH_2) 47.3 (CH) 50.8 (CH) 61.7 (CH_3) 67.1 (CH_2) 79.2 (C) 120.1 (CH) 125.3 (CH) 127.2 (CH) 127.8 (CH) 141.4 (C) 144.1 (C) 156.1 (CO) 156.3 (CO) 172.8 (CO) ppm. HRMS (ES$^+$) $C_{28}H_{37}N_3O_6Na$ (MNa^+) calcd. 534.2580; found 534.2577. $[\alpha]_D$ = –12 (*c* = 0.1, CH_2Cl_2).

***tert*-Butyl 5*S*-amino-6-(methoxy(methyl)amino)-6-oxohexylcarbamate 7.**[19] Weinreb amide **6** (150 mg, 0.29 mmol, 1 equiv.) was stirred in a 20% solution of piperidine in acetonitrile (5 mL) for 15 minutes. Reaction completion was confirmed by TLC. Excess solvent and piperidine was removed *in vacuo* to give the crude product which was purified by column chromatography (CH_2Cl_2/MeOH; 15:1 → 8:1) to give free amine **7** (65 mg, 77%) as a yellow oil. R_f = 0.2 (CH_2Cl_2/MeOH; 15:1). IR (film): ν_{max} = 3354, 2975, 2934, 2866,

1690, 1525, 1457, 1392, 1366, 1275, 1251, 1172, 991 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.31–1.56 (m, 13H, ^{t}Bu, CH_2) 1.63–1.75 (m, 2H, CH_2) 2.02 (s (br), 2H, NH_2) 3.05–3.16 (m, 2H, CH_2) 3.21 (s, 3H, CH_3) 3.71 (s, 3H, CH_3) 3.74–3.85 (m, 1H, CH) 4.65 (s (br), 1H, NH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 23.0 (CH_2) 28.4 (CH_3) 29.8 (CH_2) 32.4 (CH_3) 34.4 (CH_2) 40.4 (CH_2) 51.0 (CH) 61.5 (CH_3) 79.0 (C) 156.0 (CO) 176.5 (CO) ppm. HRMS (ES^+) $C_{13}H_{28}N_3O_4$ (MH^+) calcd. 290.2080; found 290.2070. $[\alpha]_D = -11$ (*c* = 0.1, CH_2Cl_2).

Benzyl 1-{[6-*tert*-butoxycarbonylamino-1-(methoxy(methyl)amino)-1-oxohexan-2*S*-yl]amino-1-oxo-3-phenylpropan-2*S*-yl}carbamate 9. Under nitrogen, EDCI·HCl (186 mg, 0.97 mmol, 1 equiv.) and HOBt·H_2O (149 mg, 0.97 mmol, 1 equiv.) was stirred with **8** (289 mg, 0.97 mmol, 1 equiv.) at 0 °C in CH_2Cl_2 (15 mL) for 10 minutes. Free amine **7** (281 mg, 0.97 mmol, 1 equiv.) was added as a solution in CH_2Cl_2 (5 mL) followed by DIPEA (0.26 mL, 1.46 mmol, 1.5 equiv). This was allowed to stir overnight warming slowly to room temperature. 1 M HCl (10 mL) was added followed by water (10 mL). The mixture was diluted with CH_2Cl_2 (20 mL) and the aqueous layer was re-extracted with CH_2Cl_2 (2 × 20 mL). The combined organic layers were dried over $MgSO_4$, filtered and solvent was removed *in vacuo* to give the crude product. Purification by column chromatography (*c*–Hex/EtOAc; 1:1) yielded dipeptide Weinreb amide **9** (334 mg, 60%) as a white foam. M.p. = 47–50 °C. R_f = 0.5 (*c*–Hex/EtOAc 1:3). IR (film): ν_{max} = 3325, 2932, 2863, 1706, 1648, 1528, 1455, 1393, 1250, 1173 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.20–1.31 (m, 2H, CH_2) 1.38–1.60 (m, 11H, ^{t}Bu, CH_2) 1.65–1.78 (m, 2H, CH_2) 2.98–3.13 (m, 4H, CH_2) 3.18 (s, 3H, CH_3) 3.73 (s, 3H, CH_3) 4.42–4.52 (m, 1H, CH) 4.71 (s (br), 1H, NH) 4.86–4.99 (m, 1H, CH) 5.03–5.12 (m, 2H, CH_2) 5.40 (s (br), 1H, NH) 6.66 (d, *J* = 7.5 Hz, 1H, NH) 7.12–7.38 (m, 10H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.4 (CH_2) 28.6 (CH_3) 29.4 (CH_2) 32.15 (CH_2) 32.25 (CH_3) 38.5 (CH_2) 40.3 (CH_2) 49.0 (CH) 56.2 (CH) 61.7 (CH_3) 67.1 (CH_2) 79.2 (C) 127.1 (CH) 128.2 (CH) 128.3 (CH) 128.6 (CH) 128.7 (CH) 129.5 (CH) 136.3 (C) 136.4 (C) 156.0 (CO) 156.1 (CO) 170.8 (CO) 172.0 (CO) ppm. HRMS (ES^+) $C_{30}H_{42}N_4O_7Na$ (MNa^+) calcd. 593.2951; found 593.2961. $[\alpha]_D = -11$ (*c* = 0.1, CH_2Cl_2).

2*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-6-(*tert*-butoxycarbonyl amino)hexanal 10.[3] Lithium aluminium hydride (134 mg, 3.53 mmol, 4 equiv.) was stirred in THF (20 mL) at –78 °C, under nitrogen. Weinreb amide **9** (503 mg, 0.88 mmol, 1 equiv.), in THF (5 mL), was added and the mixture stirred for 5 minutes. A 1 M solution of $KHSO_4$ (~ 10 mL) was cautiously added dropwise at –78 °C. Ethyl acetate (20 mL) was then added and the solution was allowed to warm to room temperature and stirred for 30 minutes. The

organic layer was separated and the aqueous layer was re-extracted with ethyl acetate (3 × 20 mL). The combined organic layers were successively washed with 0.5 M HCl (2 × 20 mL), saturated $NaHCO_3$ (2 × 20 mL), brine (20 mL) and dried over $MgSO_4$ and filtered. Solvent was removed *in vacuo* to give aldehyde **10** (448 mg, 99%) as a white solid. R_f = 0.5 (*c*–Hex/EtOAc 1:2) with data as reported.[3]

***trans*-3*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-1-(phenylsulfonyl)hept-1-en-7-*tert*-butyloxycarbonylamine 4.** At 0 °C, under nitrogen, 60% sodium hydride in mineral oil (31 mg, 0.79 mmol, 1.2 equiv.) was stirred in THF (4 mL). Phosphonate **11** (272 mg, 0.93 mmol, 1.4 equiv) was added dropwise as a solution in THF (4 mL) and the reaction mixture was stirred for 30 minutes. Aldehyde **10** (339 mg, 0.66 mmol, 1 equiv) was then added as a solution in THF (6 mL). The reaction was left to stir overnight gradually warming to room temperature. The reaction mixture was quenched with water (10 mL) and the layers were partitioned with dichloromethane (10 mL). The aqueous layer was re-extracted with dichloromethane (2 × 10 mL) and the combined organic layers were washed with brine (15 mL) and dried over $MgSO_4$. The solution was filtered and solvent was removed *in vacuo* to give the crude product which was purified by column chromatography (*c*–Hex/EtOAc; 1:1) to give vinyl sulfone **4** (336 mg, 78%) as a white solid. R_f = 0.3 (*c*–Hex/EtOAc 1:1). With data as reported.[3]

Benzyl 1-{[6-amino-1-(methoxy(methyl)amino)-1-oxohexan-2*S*-yl]amino-1-oxo-3-phenylpropan-2*S*-yl}carbamate 12. Weinreb amide **9** (293 mg, 0.51 mmol, 1 equiv.) was stirred in a 70% solution of TFA in CH_2Cl_2 (3 mL) for 1 hour. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and was washed with sat. aq. $NaHCO_3$ (2 × 5 mL), brine (10 mL) and dried over $MgSO_4$. Filtration, followed by solvent removal *in vacuo* gave amine **12** as a yellow oil (218 mg, 91%) which was used without futher purification. IR (film): ν_{max} = 3294, 2939, 1704, 1647, 1535, 1499, 1456, 1394, 1264, 1053, 749 cm^{-1}. HRMS (ES^+) $C_{25}H_{35}N_4O_5$ (MH^+) calcd. 471.2607; found 471.2613. $[\alpha]_D$ = –8 (*c* = 0.1, CH_2Cl_2).

Benzyl 1-{[(6-naphthylsulfonylamino-1-(methoxy(methyl)amino)-1-oxohexan-2*S*-yl)amino]-1-oxo-3-phenylpropan-2*S*-yl}carbamate 13. At 0 °C, triethylamine (0.02 mL, 0.16 mmol, 1.1 equiv.) was added to a stirred solution of amine **12** (64 mg, 0.14 mmol, 1 equiv.) and 1-naphthalene sulfonyl chloride (34 mg, 0.16 mmol, 1.1 equiv.) in anhydrous CH_2Cl_2 (5 mL). The reaction was left to stir overnight warming slowly to room temperature. Water was added (5 mL) and the layers partitioned. The aqueous layer was re-extracted with

CH_2Cl_2 (2 x 10 mL) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered and solvent was removed *in vacuo*. The residue was purified by column chromatography (*c*–Hex/EtOAc; 1:1) to give sulfonamide **13** (89 mg, 99%) as a white solid. M.p. = 62–65 °C. R_f = 0.3 (*c*–Hex/EtOAc; 1:2). IR (film): ν_{max} = 3300, 3063, 2926, 2857, 1708, 1646, 1537, 1509, 1455, 1323, 1265, 1240, 1161, 1134, 1086 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$): δ = 1.17–1.45 (m, 4H, CH_2) 1.53–1.76 (m, 2H, CH_2) 2.78–2.91 (m, 2H, CH_2) 3.03 (dd, *J* = 14.0, 8.0 Hz, 1H, CH_2) 3.07–3.20 (m, 4H, CH_3, CH_2) 3.66 (s, 3H, CH_3) 4.47 (app. q, *J* = 7.0 Hz, 1H, CH) 4.83–4.91 (m, 1H, CH) 5.00–5.10 (m, 2H, CH_2) 5.44 (s, 1H, NH) 5.53 (d, *J* = 8.0 Hz, 1H, NH) 6.75 (d, *J* = 8.0 Hz, 1H, NH) 7.12–7.34 (m, 10H, ArH) 7.48–7.53 (m, 1H, ArH) 7.54–7.59 (m, 1H, ArH) 7.60–7.64 (m, 1H, ArH) 7.92 (d, *J* = 8.0 Hz, 1H, ArH) 8.03 (d, *J* = 8.0 Hz, 1H, ArH) 8.23 (dd, *J* = 7.5, 1.0 Hz, 1H, ArH) 8.69 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 21.7 (CH_2) 28.6 (CH_2) 31.8 (CH_2) 32.2 (CH_3) 38.2 (CH_2) 42.6 (CH_2) 48.6 (CH) 56.2 (CH) 61.6 (CH_3) 67.0 (CH_2) 124.1 (CH) 124.6 (CH) 126.8 (CH) 126.9 (CH) 127.9 (CH) 128.0 (CH) 128.1 (C) 128.2 (CH) 128.4 (CH) 128.6 (CH) 129.0 (CH) 129.3 (CH) 129.4 (CH) 134.0 (CH) 134.3 (C) 135.0 (C) 136.2 (C) 136.3 (C) 156.1 (CO) 171.0 (CO) 171.1 (CO) ppm. HRMS (ES^+) $C_{35}H_{40}N_4O_7NaS$ (MNa^+) calcd. 683.2515; found 683.2537. $[\alpha]_D$ = –7 (*c* = 0.1, CH_2Cl_2).

Benzyl 1-{[(6-azido-1-(methoxy(methyl)amino)-1-oxohexan-2*S*-yl)amino]-1-oxo-3-phenylpropan-2*S*-yl}carbamate 15. Imidazole-1-sulfonyl azide hydrochloride **14** (70mg, 0.33 mmol, 1.2 equiv.) was added to a stirred solution of amine **12** (130 mg, 0.28 mmol, 1 equiv.), K_2CO_3 (58 mg, 0.42 mmol, 1.5 equiv.) and $CuSO_4 \cdot 5H_2O$ (1 mg, 0.003 mmol, 0.01 equiv) in MeOH (3 mL). The reaction mixture was left to stir overnight at room temperature. Solvent was removed *in vacuo* and water (2 mL) was added to the residue which was stirred again. Conc. HCl (3 mL) was added dropwise and the mixture extracted with EtOAc (3 x 10 mL). The combined organic layers were dried over $MgSO_4$, filtered and solvent was removed *in vacuo* to give an oily, crude residue. Purification by silica gel column chromatography (*c*–Hex/EtOAc; 2:1) gave azide **15** (52 mg, 38%) as a white crystalline solid. M.p. = 49–51 °C. R_f = 0.7 (*c*–Hex/EtOAc; 1:2). IR (film): ν_{max} = 3294, 2940, 2097, 1938, 1719, 1646, 1533, 1443, 1258 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.24–1.37 (m, 2H, CH_2) 1.47–1.79 (m, 4H, CH_2) 3.01–3.15 (m, 2H, CH_2) 3.16–3.25 (m, 5H, CH_3, CH_2) 3.74 (s, 3H, CH_3) 4.41–4.50 (m, 1H, CH) 4.86–4.97 (m, 1H, CH) 5.09 (s, 2H, CH_2) 5.27 (d, *J* = 6.5 Hz, 1H, NH) 6.53 (d, *J* = 7.5 Hz, 1H, NH) 7.13–7.39 (m, 10H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.3 (CH_2) 28.4 (CH_2) 32.0 (CH_3, CH_2) 38.4 (CH_2) 48.9 (CH) 51.1 (CH_2)

56.1 (CH) 61.7 (CH_3) 67.1 (CH_2) 127.0 (CH) 128.0 (CH) 128.2 (CH) 128.5 (CH) 128.7 (CH) 129.3 (CH) 136.1 (2 × C) 155.8 (CO) 170.5 (CO) 171.6 (CO) ppm. HRMS (ES^+) $C_{25}H_{32}N_6O_5Na$ (MNa^+) calcd. 519.2332; found 519.2323. $[\alpha]_D$ = –13 (*c* = 0.1, CH_2Cl_2). *Note: Standard precautions were taken when working with azides. No metal spatulas were used and a safety shield was placed in front of the reaction vessels. Although the hydrochloride salt of imidazole-1-sulfonyl azide was prepared and stored (–20 °C) without issue in our hands, it has recently come to light that the hydrochloride salt can decompose to hydrazoic acid, particularly if moisture is present, which can be explosive.*[11(b)] *Thus the hydrogen sulfate, or tetrafluoroborate salt, are now strongly recommended due to their enhanced stability and impact insensitivity.*[11(c)] *Also a safer method to access this diazotransfer reagent has been developed by Wang, Shen and co-workers.*[20]

***trans*-3*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-1-(phenylsulfonyl)hept-1-en-7-(2-naphthalene)sulfonamide 17.** Ammonium salt **16** (40 mg, 0.06 mmol, 1 equiv.) was stirred with 2-naphthalenesulfonyl chloride (64 mg, 0.28 mmol, 5 equiv.) in CH_2Cl_2 (1 mL). Triethylamine (50 μL, 0.36 mmol, 6 equiv.) was added and the reaction was left to stir overnight. The reaction mixture was diluted in CH_2Cl_2 (5 mL) and washed with water (5 mL). The aqueous layer was re-extracted with CH_2Cl_2 (4 × 5 mL). The combined organic layers washed with brine (5 mL), dried over $MgSO_4$, and filtered. Solvent was removed *in vacuo* to give the crude product, which was purified by column chromatography (*c*–Hex/EtOAc; 1:1) to give sulfonamide **17** (26 mg, 60%) as a white solid. M.p. = 152–155 °C. R_f = 0.3 (*c*–Hex/EtOAc; 1:1). IR (film): ν_{max} 3335, 3288, 3058, 2948, 2870, 1699, 1658, 1524, 1446, 1325, 1253, 1155, 1072, 750 cm^{-1}. ^1H NMR (400 MHz, d^6–DMSO): δ = 1.01–1.56 (m, 6H, CH_2) 2.59–2.75 (m, 3H, CH_2) 2.83–2.91 (m, 1H, CH_2) 4.12–4.21 (m, 1H, CH) 4.35–4.44 (m, 1H, CH) 4.82–4.94 (m, 2H, CH_2) 6.35 (d, *J* = 15.0 Hz, 1H, CH) 6.76 (dd, *J* = 15.0, 5.0 Hz, 1H, CH) 7.05–7.31 (m, 9H, ArH) 7.49 (d, *J* = 8.0 Hz, 1H, NH) 7.57–7.73 (m, 5H, ArH + NH) 7.75–7.86 (m, 4H, ArH) 8.02 (d, *J* = 8.0 Hz 1H, NH) 8.04–8.17 (m, 4H ArH) 8.39–8.43 (m, 1H ArH) ppm. ^{13}C NMR (100 MHz, d^6–DMSO): δ = 22.3 (CH_2) 28.6 (CH_2) 32.5 (CH_2) 37.4 (CH_2) 42.4 (CH_2) 48.9 (CH) 56.2 (CH) 65.2 (CH_2) 122.2 (CH) 126.3 (CH) 127.1 (CH) 127.2 (CH) 127.49 (CH) 127.53 (CH) 127.7 (CH) 127.8 (CH) 128.0 (CH) 128.2 (CH) 128.6 (CH) 129.1 (CH) 129.3 (CH) 129.6 (CH) 131.7 (C) 133.6 (CH) 134.1 (C) 136.9 (C) 137.5 (C) 137.6 (C) 140.3 (C) 147.2 (CH) 155.7 (CO) 171.1 (CO) ppm. *Note: 2 coincident peaks in ^{13}C NMR spectrum*. HRMS (ES^+) $C_{40}H_{41}N_3O_7NaS_2$ (MNa^+) calcd. 762.2284; found 762.2319. $[\alpha]_D$ = –12.0 (*c* = 0.1, Me_2CO).

***trans*-3*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-1-(phenylsulfonyl)hept-1-en-7-hexylsulfonamide 18**. Ammonium salt **16** (30 mg, 0.05 mmol, 1 equiv.) was stirred with 1-hexanesulfonyl chloride (41 mg, 0.23 mmol, 5 equiv.) in CH_2Cl_2 (1 mL). Triethylamine (38 μL, 0.27 mmol, 6 equiv.) was added and the reaction the reaction was left to stir overnight. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with water (5 mL). The aqueous layer was re-extracted with CH_2Cl_2 (4 × 5 mL) and the combined organic layers washed with brine (5 mL), dried over $MgSO_4$, and filtered. Solvent was removed *in vacuo* to give the crude product, which was purified by column chromatography (*c*–Hex/EtOAc; 1:1) to give sulfonamide **18** (24 mg, 76%) as a white solid. M.p. = 220–222 °C (decomp.). R_f = 0.4 (*c*–Hex/EtOAc; 1:1). IR (film): ν_{max} 3312, 3065, 2932, 2860, 1703, 1664, 1530, 1455, 1318, 1258, 1146, 1086, 1037 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 0.88 (t, *J* = 7.0 Hz, 3H, CH_3) 1.22–1.79 (m, 14H, CH_2) 2.90–3.08 (m, 6H, CH_2) 4.40 (app. q, *J* = 7.5 Hz, 1H, CH) 4.59–4.67 (m, 1H, CH) 4.72–4.79 (m, 1H, NH) 5.03 (s, 2H, CH_2) 5.68 (d, *J* = 8.0 Hz, 1H, NH) 6.07 (d, *J* = 15.0 Hz, 1H, CH) 6.32 (d, *J* = 8.5 Hz, 1H, NH) 6.79 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.11–7.21 (m, 5H, ArH) 7.23–7.36 (m, 5H, ArH) 7.55 (t, *J* = 8.0 Hz, 2H, ArH) 7.63 (t, *J* = 7.5 Hz, 1H, ArH) 7.85 (d, *J* = 8.0 Hz, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 14.1 (CH_3) 21.7 (CH_2) 22.5 (CH_2) 23.7 (CH_2) 28.1 (CH_2) 29.1 (CH_2) 31.4 (CH_2) 33.0 (CH_2) 38.3 (CH_2) 42.4 (CH_2) 49.2 (CH) 52.8 (CH_2) 56.7 (CH) 67.2 (CH_2) 127.3 (CH) 127.8 (CH) 128.1 (CH) 128.3 (CH) 128.7 (CH) 128.9 (CH) 129.4 (CH) 129.5 (CH) 130.4 (CH) 133.7 (CH) 136.2 (C) 136.4 (C) 140.2 (C) 145.8 (CH) 156.5 (CO) 171.5 (CO). HRMS (ES^+) $C_{36}H_{47}N_3O_7NaS_2$ (MNa^+) calcd. 720.2753; found 720.2717. $[\alpha]_D$ = –16.0 (*c* = 0.1, CH_2Cl_2).

***trans*-3*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-1-(phenylsulfonyl)hept-1-en-7-[1-(5-dimethylamino)naphthalene]sulfonamide 19.** Compound **4** (82 mg, 0.13 mmol, 1 equiv.) was stirred in a 70% solution of TFA in dichloromethane (3 mL) for 1 hour, after which, solvent was removed *in vacuo* to give the crude trifluoroacetate salt **16** which was taken up in CH_2Cl_2 (1 mL). The solution was cooled to 0 °C and dansyl chloride (170 mg, 0.63 mmol, 5 equiv.) was added, followed by triethylamine (0.14 mL, 1 mmol, 8 equiv.). The reaction was left to stir overnight, warming slowly to room temperature. The reaction mixture was diluted with CH_2Cl_2 (5 mL) and water (5 mL), the layers partitioned and the aqueous layer re-extracted with CH_2Cl_2 (2 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over $MgSO_4$, filtered and solvent was removed *in vacuo* to give the crude product. Purification by silica gel column chromatography (*c*–Hex/EtOAc;

1:1) gave sulfonamide **19** (91 mg, 92%) as a yellow solid. M.p. = 56–58 °C. R_f = 0.3 (*c*–Hex/EtOAc; 1:1). IR (film): ν_{max} = 3303, 3061, 2940, 2862, 2791, 1706, 1664, 1534, 1455, 1308, 1233, 1145, 1085 cm^{-1}. UV–Vis: λ_{max} abs ($CHCl_3$): 255, 338 nm. λ_{max} fluo ($CHCl_3$): 496 nm. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.15–1.45 (m, 6H, CH_2) 2.69–2.79 (m, 1H, CH_2) 2.78–2.93 (m, 7H, CH_2, CH_3) 2.99 (dd, *J* = 12.0, 5.5 Hz, 1H, CH_2) 3.03 (dd, *J* = 12.0, 6.0 Hz, 1H, CH_2) 4.47 (app. q, *J* = 7.5 Hz, 1H, CH) 4.51–4.58 (m, 1H, CH) 4.99 (app. q, *J* = 12.5 Hz, 2H, CH_2) 5.61 (s, 1H, NH) 5.75 (d, *J* = 7.5 Hz, 1H, NH) 6.03 (d, *J* = 15.0 Hz, 1H, CH) 6.42 (d, *J* = 8.0 Hz, 1H, NH) 6.74 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.09–7.18 (m, 6H, ArH) 7.19–7.30 (m, 5H, ArH) 7.44–7.56 (m, 4H, ArH) 7.60 (t, *J* = 7.5 Hz, 1H, ArH) 7.83 (d, *J* = 7.5 Hz, 2H, ArH) 8.18 (d, *J* = 6.5 Hz, 1H, ArH) 8.32 (d, *J* = 8.5 Hz, 1H, ArH) 8.51 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.9 (CH_2) 28.4 (CH_2) 33.0 (CH_2) 38.4 (CH_2) 42.4 (CH_2) 45.4 (CH_3) 49.2 (CH) 56.6 (CH) 67.0 (CH_2) 115.2 (CH) 118.9 (CH) 123.2 (CH) 127.1 (CH) 127.6 (CH) 127.8 (CH) 128.1 (CH) 128.3 (CH) 128.5 (CH) 128.7 (CH) 129.2 (CH) 129.3 (CH) 129.4 (CH) 129.6 (C) 129.9 (C) 130.1 (CH) 130.3 (CH) 133.5 (CH) 134.9 (C) 136.1 (C) 136.2 (C) 140.1 (C) 145.9 (CH) 151.9 (C) 156.3 (CO) 171.3 (CO) ppm. HRMS: $C_{42}H_{47}N_4O_7S_2$ (MH^+) calcd. 783.2886; found 783.2897. $[\alpha]_D$ = –7 (*c* = 0.1, CH_2Cl_2).

Benzyl {1-oxo-3-phenyl-1-[((2*R*-(phenylsulfonyl)methyl)azepan-3*S*-yl)amino]propan-2*S*-yl}carbamate 20 and Benzyl {1-oxo-3-phenyl-1-[((2*S*-(phenylsulfonyl)methyl)azepan-3*S*-yl)amino]propan-2-yl}carbamate 21. Ammonium salt **16** (29 mg, 0.044 mmol, 1 equiv.) was stirred in MeOH (3.5 mL). Et_3N (34 μL, 0.244 mmol, 5.5 equiv.) was added as a solution in MeOH (1 mL). The reaction mixture was left to stir for 7 days at room temperature. Solvent was removed *in vacuo* to give the crude product which was purified by flash column chromatography (EtOAc) to give azepane **20** (11 mg, 46%) as a waxy solid. Further elution gave the second diastereomer **21** (6 mg, 25%) as a white solid.

Data for 20: M.p. <40 °C. R_f = 0.8 (EtOAc). IR (film): ν_{max} = 3321, 3062, 3032, 2931, 2860, 1963, 1896, 1809, 1712, 1658, 1585, 1518, 1446, 1394, 1304, 1254, 1149, 1086, 1028, 910, 847, 741, 698 cm^{-1}. ^1H NMR (600 MHz, $CDCl_3$): δ = 1.37–1.46 (m, 2H, CH_2) 1.47–1.55 (m, 2H, CH_2) 1.64–1.75 (m, 2H, CH_2) 2.51–2.59 (m, 1H, CH_2) 2.77 (d, *J* = 14.5 Hz, 1H, CH_2) 2.79–2.86 (m, 2H, CH_2) 2.95 (dd, *J* = 13.5, 8.0 Hz, 1H, CH_2) 3.11 (dd, *J* = 13.5, 6.0 Hz, 1H, CH_2) 3.23 (d, *J* = 10.5 Hz, 1H, CH) 3.74–3.78 (m, 1H, CH) 4.33 (app. q, *J* = 7.0 Hz, 1H, CH) 5.07 (d, *J* = 12.0 Hz, 1H, CH_2) 5.14 (d, *J* = 12.0 Hz, 1H, CH_2) 5.28 (d, *J* = 6.0 Hz, 1H, NH) 6.76 (d, *J* = 7.5 Hz, 1H, NH) 7.10 (t, *J* = 7.0 Hz, 1H, ArH) 7.14–7.17 (m, 3H, ArH) 7.19 (d, *J* = 7.5 Hz, 1H, ArH) 7.31–7.39 (m, 5H, ArH) 7.61 (t, *J* = 7.5 Hz, 2H, ArH) 7.70 (t, *J* = 7.5 Hz,

1H, ArH) 7.88 (d, J = 7.5 Hz, 2H, ArH) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 20.6 (CH_2) 28.8 (CH_2) 34.2 (CH_2) 38.6 (CH_2) 47.1 (CH_2) 52.5 (CH) 54.7 (CH) 56.9 (CH) 59.1 (CH_2) 67.3 (CH_2) 127.4 (CH) 128.0 (CH) 128.3 (CH) 128.4 (CH) 128.7 (CH) 129.0 (CH) 129.3 (CH) 129.6 (CH) 134.2 (CH) 136.2 (C) 136.4 (C) 139.6 (C) 155.9 (CO) 170.1 (CO) ppm. HRMS (ES^+): $C_{30}H_{36}N_3O_5S$ (MH^+) calcd. 550.2376; found 550.2385. $[\alpha]_D$ = +32 (c = 0.1, CH_2Cl_2).

Data for 21: M.p. = 179–180 °C (decomp.) R_f = 0.6 (EtOAc). IR (film): ν_{max} = 3290, 2924, 2844, 1691, 1647, 1537, 1452, 1390, 1300, 1286, 1257, 1142, 1082, 1039, 746 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.38–1.86 (m, 6H, CH_2) 2.55–2.63 (m, 1H, CH_2) 2.91–2.98 (m, 2H, CH, CH_2) 3.00 (dd, J = 14.0, 7.0 Hz, 1H, CH_2) 3.08 (dd, J = 14.0, 6.5 Hz, 1H, CH_2) 3.11–3.20 (m, 2H, CH_2) 3.70–3.78 (m, 1H, CH) 4.28 (app. q, J = 7.0 Hz, 1H, CH) 5.09 (d, J = 12.0 Hz, 1H, CH_2) 5.12 (d, J = 12.0 Hz, 1H, CH_2) 5.16 (d, J = 6.0 Hz, 1H, NH) 6.01 (d, J = 9.0 Hz, 1H, NH) 7.18 (d, J = 7.0 Hz, 2H, ArH) 7.24–7.38 (m, 8H, ArH) 7.55 (t, J = 7.5 Hz, 2H, ArH) 7.65 (t, J = 7.5 Hz, 1H, ArH) 7.90 (d, J = 7.5 Hz, 2H, ArH) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 22.3 (CH_2) 30.3 (CH_2) 32.7 (CH_2) 38.0 (CH_2) 47.3 (CH_2) 54.6 (CH) 56.5 (CH) 58.7 (CH_2) 59.3 (CH) 67.4 (CH_2) 127.3 (CH) 127.9 (CH) 128.30 (CH) 128.33 (CH) 128.6 (CH) 128.9 (CH) 129.26 (CH) 129.32 (CH) 133.8 (CH) 136.0 (C) 136.1 (C) 139.8 (C) 155.9 (CO) 170.3 (CO) ppm. HRMS (ES^+): $C_{30}H_{36}N_3O_5S$ (MH^+) calcd. 550.2376; found 550.2376. $[\alpha]_D$ = +6 (c = 0.1, CH_2Cl_2).

Data for trifluoroacetamide 22. M.p. = 145–147 °C. R_f = 0.7 (*c*-Hex/EtOAc 1:2). IR (film): ν_{max} 3310, 2934, 1708, 1663, 1539, 1501, 1447, 1307, 1211, 1183, 1146, 1086 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): 1.18–1.72 (m, 6H, CH_2) 2.98 (dd, J = 12.5, 6.5 Hz, 1H, CH_2) 3.03 (dd, J = 12.5, 5.5 Hz, 1H, CH_2) 3.08–3.21 (m, 1H, CH_2) 3.25–3.38 (m, 1H, CH_2) 4.29 (app. q, J = 7.5 Hz, 1H, CH) 4.58–4.69 (m, 1H, CH) 5.05 (s, 2H, CH_2) 5.37 (d, J = 7.0 Hz, 1H, NH) 5.95–6.04 (m, 2H, CH, NH) 6.74 (dd, J = 15.0, 4.5 Hz, 1H, CH) 6.87 (s (br), 1H, NH) 7.09–7.38 (m, 10H, ArH) 7.56 (t, J = 7.5 Hz, 2H. ArH) 7.64 (t, J = 7.5 Hz, 1H, ArH) 7.84 (d, J = 7.5 Hz, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.2 (CH_2) 28.1 (CH_2) 33.2 (CH_2) 38.1 (CH_2) 39.3 (CH_2) 49.0 (CH) 56.8 (CH) 67.2 (CH_2) 115.9 (q, J_{CF} = 288 Hz, CF_3) 127.4 (CH) 127.7 (CH) 127.9 (CH) 128.3 (CH) 128.6 (CH) 128.9 (CH) 129.1 (CH) 129.3 (CH) 130.5 (CH) 133.6 (CH) 135.86 (C) 135.91 (C) 139.9 (C) 145.2 (CH) 156.1 (CO) 157.5 (q, J_{CF} = 37 Hz, CO) 171.0 (CO) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = –75.65 ppm. HRMS (ES^+) $C_{32}H_{34}N_3O_6NaSF_3$ (MNa^+) calcd. 668.2018; found 668.2006. $[\alpha]_D$ = –22 (c = 0.1, MeOH).

***trans*-7-(Naphthalene-1-sulfonamido)-1-(phenylsulfonyl)hept-1-en-3*S*-yl)amino)-1-oxo-3-phenylpropan-2*S*-ammonium bromide 23**. Compound **1** (492 mg, 0.66 mmol, 1 equiv.) was stirred in CH_2Cl_2 (3 mL) at 0 °C before 33% HBr, in AcOH (3 mL) was added dropwise and the reaction mixture was left to stir for 2 h whilst warming gradually to room temperature. Solvent was removed *in vacuo* and the crude product was re-dissolved in the minimum amount of CH_2Cl_2 (~ 0.5 mL). Et_2O (30 mL) was added and the precipitate was stirred for 10 minutes. The suspension was allowed to settle and the supernatant was removed. This process was repeated three times. The residue was dried under high vacuum to give ammonium salt **23** (438 mg, 96%) as an off white powder. M.p. = 116–120 °C (decomp). IR (film): ν_{max} = 3056, 2936, 1679, 1553, 1505, 1447, 1307, 1146, 1085, 1045 cm^{-1}. ^1H NMR (400 MHz, d^6–DMSO): δ = 1.02–1.20 (m, 2H, CH_2) 1.22–1.45 (m, 4H, CH_2) 2.66–2.77 (m, 2H, CH_2) 2.92–3.02 (m, 2H, CH_2) 3.89–4.00 (m, 1H, CH) 4.29–4.38 (m, 1H, CH) 6.15 (dd, *J* = 15.0, 1.5, 1H, CH) 6.66 (dd, *J* = 15.0, 5.0 Hz, 1H, CH) 7.10–7.38 (m, 7H, ArH) 7.61–7.77 (m, 5H, ArH) 7.92 (t, *J* = 5.5 Hz, 1H, NH) 8.06–8.12 (m, 3H, ArH) 8.16–8.30 (m, 4H, ArH, NH_3) 8.43 (d, *J* = 8.0 Hz, 1H, NH) 8.65 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, d^6–DMSO): δ = 22.1 (CH_2) 28.7 (CH_2) 32.4 (CH_2) 36.9 (CH_2) 42.1 (CH_2) 49.2 (CH) 53.4 (CH) 124.5 (CH) 124.7 (CH) 126.9 (CH) 127.2 CH) 127.5 (CH) 127.8 (CH) 128.3 (C) 128.4 (CH) 128.5 (CH) 128.9 (CH) 129.3 (CH) 129.5 (CH) 129.6 (CH) 133.6 (CH) 133.7 (CH) 133.8 (C) 134.6 (C) 135.6 (C) 140.1 (C) 146.1 (CH) 164.7 (CO) ppm. HRMS (ES^+) $C_{32}H_{31}N_4O_5NaS$ (MNa^+) calcd. 606.1913; found 606.1926. $[\alpha]_D$ = –11 (*c* = 0.1, MeOH).

***N*-[5*S*-Benzyl-6-oxo-3*R*-(phenylsulfonylmethyl)piperazin-2*S*-yl]butyl-(1-naphthalene) sulfonamide 24.** Ammonium bromide salt **23** (30 mg, 0.044 mmol, 1 equiv.) was stirred in MeOH (2.5 mL). Et_3N (32 μL, 0.230 mmol, 5.2 equiv.) was added as a solution in MeOH (1 mL) and the reaction mixture was left to stir at rt for 24 h. Solvent was removed *in vacuo* and the crude product was purified by column chromatography (EtOAc) to give adduct **24** (15 mg, 57%) as a white solid. M.p. = 67–71 °C. R_f = 0.4 (EtOAc). ^1H NMR (600 MHz, $CDCl_3$): δ = 1.06–1.19 (m, 4H, CH_2) 1.22–1.31 (m, 2H, CH_2) 2.79–2.88 (m, 2H, CH_2) 3.00 (dd, *J* = 13.5, 7.5 Hz, 1H, CH_2) 3.02–3.05 (m, 1H, CH) 3.06 (dd, *J* = 13.5, 3.5 Hz, 1H, CH_2) 3.15 (dd, *J* = 14.0, 5.0 Hz, 1H, CH_2) 3.33 (dd, *J* = 14.0, 7.5 Hz, 1H, CH_2) 3.42–3.47 (m, 1H, CH) 3.68–3.74 (m, 1H, CH) 5.53 (t, *J* = 6.0 Hz, 1H, NH) 6.68 (s, 1H, NH) 7.15–7.22 (m, 3H, ArH) 7.23–7.27 (m, 2H, ArH) 7.49–7.56 (m, 3H, ArH) 7.58 (t, *J* = 7.5 Hz, 1H, ArH) 7.60–7.69 (m, 2H, ArH) 7.84 (d, *J* = 7.5 Hz, 2H, ArH) 7.94 (d, *J* = 8.0 Hz, 1H, ArH) 8.07 (d, *J* =

8.0 Hz, 1H, ArH) 8.24 (dd, *J* = 7.5, 1.0 Hz, 1H, ArH) 8.66 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 21.9 (CH_2) 28.9 (CH_2) 34.1 (CH_2) 38.2 (CH_2) 42.7 (CH_2) 48.2 (CH) 55.3 (CH) 55.6 (CH) 57.2 (CH_2) 124.2 (CH) 124.4 (CH) 126.8 (CH) 126.9 (CH) 127.7 (CH) 128.2 (C) 128.3 (CH) 128.5 (CH) 129.1 (CH) 129.4 (CH) 129.6 (CH) 129.8 (CH) 134.0 (CH) 134.2 (CH) 134.3 (C) 134.7 (C) 137.2 (C) 139.6 (C) 171.1 (CO) ppm. HRMS (ES^+): $C_{32}H_{35}N_3O_5S_2Na$ (MNa) calcd. 628.1916; found 628.1930. $[\alpha]_D$ = –24 (*c* = 0.1, CH_2Cl_2).

***trans*-Benzyl 7-{[(((2-hydroxybenzylidene)amino)-1-(phenylsulfonyl)hept-1-en-3*S*-yl)amino]-1-oxo-3-phenylpropan-2*S*-yl}carbamate 25.** Salicylaldehyde **27** (5 μL, 0.05 mmol, 1.1 equiv.) was stirred with ammonium salt **16** (29 mg, 0.04 mmol, 1 equiv.) in CH_2Cl_2 (0.5 mL). Triethylamine (24 μL, 0.17 mmol, 4 equiv.) was added to give a bright yellow solution which was stirred for 5 minutes. Solvent was removed *in vacuo* to give the crude product which was purified by silica gel column chromatography (*c*–Hex/EtOAc; 1:1) to give imine **25** as a yellow solid (20 mg, 70%). M.p. = 110–114 °C. R_f = 0.3 (*c*–Hex/EtOAc; 1:1). IR (film): ν_{max} = 3300, 3063, 3033, 2926, 2855, 1707, 1661, 1633, 1584, 1539, 1498, 1455, 1452, 1307, 1281, 1260, 1146, 1086 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.21–1.74 (m, 6H, CH_2) 3.00 (dd, *J* = 14.0, 7.0 Hz, 1H, CH_2) 3.07 (dd, *J* = 14.0, 7.0 Hz, 1H, CH_2) 3.47–3.58 (m, 2H, CH_2) 4.39 (app. q, *J* = 7.5 Hz, 1H, CH) 4.63–4.72 (m, 1H, CH) 5.04 (s, 2H, CH_2) 5.67 (d, *J* = 6.5 Hz, 1H, NH) 5.96 (d, *J* = 8.5 Hz, 1H, NH) 6.17 (dd, *J* = 15.0, 1.5 Hz, 1H, CH) 6.78 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 6.83–6.88 (m, 1H, ArH) 6.90 (d, *J* = 8.0 Hz, 1H, ArH) 7.10–7.28 (m, 10H, ArH) 7.30–7.35 (m, 2H, ArH) 7.50–7.57 (m, 2H, ArH) 7.59–7.65 (m, 1H, ArH) 7.85 (d, *J* = 7.5 Hz, 2H, ArH) 8.29 (s, 1H, CH) 13.73 (s (br), 1H, OH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.9 (CH_2) 29.9 (CH_2) 33.6 (CH_2) 38.0 (CH_2) 49.2 (CH) 56.6 (CH) 58.3 (CH_2) 67.2 (CH_2) 117.2 (CH) 118.55 (CH) 118.6 (C) 127.2 (CH) 127.7 (CH) 128.1 (CH) 128.3 (CH) 128.6 (CH) 128.8 (CH) 129.2 (CH) 129.3 (CH) 130.5 (CH) 131.2 (CH) 132.3 (CH) 133.5 (CH) 136.0 (C) 136.3 (C) 140.0 (C) 145.3 (CH) 156.3 (CO) 161.4 (C) 164.9 (CH) 170.7 (CO) ppm. HRMS (ES^+) $C_{37}H_{40}N_3O_6S$ (MH^+) calcd. 654.2638; found 654.2642. $[\alpha]_D$ = –9 (*c* = 0.1, CH_2Cl_2).

***trans*-Benzyl 7-{[(((((3-hydroxy-5-(hydroxymethyl)-2-methylpyridin-4-yl)methylidene)amino)-1-(phenylsulfonyl)hept-1-en-3*S*-yl)amino]-1-oxo-3-phenylpropan-2*S*-yl}carbamate 26.** Ammonium salt **16** (20 mg, 0.03 mmol, 1 equiv.) and pyridoxal hydrochloride salt **28** (6 mg, 0.03 mmol, 1 equiv.) were dissolved in distilled water (2 mL) and heated until homogeneous. The reaction mixture was stirred and Na_2CO_3 (16 mg, 0.15 mmol, 5 equiv.) was added as a solution in distilled water (1 mL) to give a bright yellow

precipitate. The reaction mixture was extracted with CH_2Cl_2 (2 × 10 mL), washed with water (5 mL), brine (5 mL) and dried over Na_2SO_4. The mixture was filtered and solvent was removed *in vacuo* to give imine **26** as a yellow solid (11 mg, 52%). *Note: attempts to further purify this imine by column chromatography (silica gel or alumina) resulted in substantial decomposition*. M.p. = 57–63 °C (decomp.). R_f = 0.2 (EtOAc). IR (film): ν_{max} = 3309, 2925, 2859, 1707, 1663, 1533, 1499, 1446, 1402, 1289, 1258, 1145, 1085, 1027, 740, 703 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.19–1.37 (m, 2H, CH_2) 1.42–1.88 (m, 4H, CH_2) 2.47 (s, 3H, CH_3) 2.96 (d, *J* = 7.5 Hz, 2H, CH_2) 3.49–3.77 (m, 2H, CH_2) 4.27 (app. q, *J* = 7.5 Hz, 1H, CH) 4.55–4.68 (m, 1H, CH) 4.72 (d, *J* = 12.5 Hz, 1H, CH_2) 4.88 (d, *J* = 12.5 Hz, 1H, CH_2) 4.98 (s, 2H, CH_2) 6.13 (d, *J* = 6.0 Hz, 1H, NH) 5.93–6.09 (m, 2H, CH, NH) 6.72 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.01–7.42 (m, 9H, ArH) 7.48–7.69 (m, 4H, ArH) 7.81 (d, *J* = 7.0 Hz, 2H, ArH) 7.90 (s, 1H, ArH) 8.78 (s, 1H, CH) 14.16 (s (br), 1H, OH) ppm. *Note: OH of benzylic alcohol not observed*. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 19.0 (CH_3) 22.5 (CH_2) 29.3 (CH_2) 33.4 (CH_2) 38.2 (CH_2) 49.3 (CH) 56.5 (CH) 58.4 (CH_2) 60.5 (CH_2) 67.2 (CH_2) 119.6 (C) 127.3 (CH) 127.6 (CH) 128.1 (CH) 128.3 (CH) 128.6 (CH) 128.8 (CH) 129.1 (CH) 129.3 (CH) 130.4 (CH) 131.2 (C) 133.6 (CH) 135.8 (C) 136.0 (C) 137.7 (CH) 139.9 (C) 145.3 (CH) 150.9 (C) 155.3 (C) 156.2 (CO) 163.1 (CH) 170.8 (CO) ppm. HRMS (ES^+) $C_{38}H_{42}N_4O_7NaS$ (MNa^+) calcd. 721.2672; found 721.2691. $[\alpha]_D$ = –16 (*c* = 0.1, CH_2Cl_2).

***trans*-5*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-7-azido-1-(phenylsulfonyl)hept-1-ene 29**. Vinyl sulfone **4** (593 mg, 0.91 mmol, 1 equiv.) was stirred in a 70% solution of trifluoroacetic acid in CH_2Cl_2 (6 mL) for 1 hour. Reaction completion was confirmed by TLC and solvent was removed *in vacuo* to give the crude trifluoroacetate salt **16**. Diazo-transfer reagent **14** (575 mg, 2.74 mmol, 3 equiv.) and $CuSO_4{\cdot}5H_2O$ (2 mg, 0.008 mmol, 0.01 equiv.) was added to the crude ammonium salt. Methanol (3 mL) was added and the mixture was stirred for 10 minutes. Potassium carbonate (750 mg, 5.42 mmol, 6 equiv.) was added and the reaction was stirred overnight. Solvent was removed *in vacuo* and water (5 mL) was added followed by dropwise addition of 5 M HCl (5 mL). The aqueous layer was extracted with ethyl acetate (3 × 10 mL), washed with brine (10 mL), dried over $MgSO_4$ and filtered. Solvent was removed *in vacuo* to give the crude product which was purified by column chromatography (*c*–Hex/EtOAc; 1:1) to give azide **29** (363 mg, 69%) as a white crystalline solid. M.p. = 114–116 °C. R_f = 0.7 (*c*–Hex/EtOAc; 1:2). IR (film): ν_{max} = 3304, 3064, 3032, 2933, 2862, 2097, 1700, 1662, 1539, 1533, 1456, 1447, 1307, 1289, 1259, 1146, 1086, 1028 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.22–1.36 (m, 2H, CH_2)

1.38–1.60 (m, 4H, CH_2) 2.98 (dd, *J* = 13.5, 8.0 Hz, 1H, CH_2) 3.08 (dd, *J* = 13.5, 6.0 Hz, 1H, CH_2) 3.20 (t, *J* = 6.5 Hz, 2H, CH_2) 4.31 (app. q, *J* = 7.5 Hz, 1H, CH) 4.57–4.66 (m, 1H, CH) 5.08 (s, 2H, CH_2) 5.26 (d, *J* = 6.5 Hz, 1H, NH) 5.78 (d, *J* = 8.0 Hz, 1H, NH) 6.08 (dd, *J* = 15.0, 1.5 Hz, 1H, CH) 6.74 (dd, *J* = 15.0, 5.0 Hz, 1H, CH) 7.10–7.15 (m, 2H, ArH) 7.17–7.25 (m, 3H, ArH) 7.29–7.40 (m, 5H, ArH) 7.52–7.59 (m, 2H, ArH) 7.62–7.67 (m, 1H, ArH) 7.85 (d, *J* = 7.0 Hz, 2H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.7 (CH_2) 28.2 (CH_2) 33.5 (CH_2) 38.4 (CH_2) 49.2 (CH) 50.9 (CH_2) 56.5 (CH) 67.1 (CH_2) 127.2 (CH) 127.6 (CH) 127.9 (CH) 128.3 (CH) 128.6 (CH) 128.8 (CH) 129.2 (CH) 129.3 (CH) 130.5 (CH) 133.6 (CH) 135.95 (C) 136.0 (C) 140.0 (C) 145.4 (CH) 156.0 (CO) 170.7 (CO) ppm. HRMS (ES^+) $C_{30}H_{33}N_5O_5NaS$ (MNa^+) calcd. 598.2100; found 598.2078. $[\alpha]_D$ = –7 (*c* = 0.1, CH_2Cl_2).

***trans*-5*S*-(2*S*-Benzyloxycarbonylamino-3-phenylpropanamido)-7-(1-benzotriazolyl)-1-(phenylsulfonyl)hept-1-ene 31.** Caesium fluoride (16 mg, 0.1 mmol, 2 equiv.) was added to a small screw cap vial containing triflate **30** (19 mg, 0.06 mmol, 1.2 equiv.) and azide **29** (30 mg, 0.05 mmol, 1 equiv.) in acetonitrile (0.75 mL). The vial was sealed and the reaction was stirred overnight at room temperature. The reaction mixture was poured onto a saturated solution of $NaHCO_3$ (2 mL) and the vial rinsed with EtOAc (5 mL).The layers were partitioned and the aqueous layer was extracted with EtOAc (2 × 5 mL) and the combined organic layers were dried over $MgSO_4$, filtered and solvent was removed *in vacuo*. The residue was purified by column chromatography (*c*-Hex/EtOAc 1:1) to give benzotriazole **31** as a white solid (29 mg, 85%). M.p. = 68–70°C. R_f = 0.4 (*c*-Hex/EtOAc 1:2). IR (film): ν_{max} = 3302, 3062, 2926, 2856, 1712, 1664, 1535, 1497, 1455, 1307, 1288, 1245, 1146, 1086 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.12–1.35 (m, 2H, CH_2) 1.46–1.69 (m, 2H, CH_2) 1.83–2.05 (m, 2H, CH_2) 2.93 (dd, *J* = 13.5, 7.5 Hz, 1H, CH_2) 3.04 (dd, *J* = 13.5, 7.0 Hz, 1 H, CH_2) 4.38 (app. q, *J* = 7.5 Hz, 1H, CH) 4.50–4.65 (m, 3H, CH_2 + CH) 5.04 (s, 2H, CH_2) 5.88 (d, *J* = 7.5 Hz, 1H, NH) 6.19 (dd, *J* = 15.0, 1.5 Hz, 1H, CH) 6.37 (d, *J* = 8.5 Hz, 1H, NH) 6.77 (dd, *J* = 15.0, 4.5 Hz, 1H, CH) 7.12–7.21 (m, 5H, ArH) 7.12–7.33 (m, 5H, ArH) 7.34–7.39 (m, 1H, ArH) 7.45–7.57 (m, 4H. ArH) 7.58–7.64 (m, 1H, ArH) 7.84 (d, *J* = 7.5 Hz, 2H, ArH) 8.02 (d, *J* = 8.5 Hz, 1H, ArH) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 22.1 (CH_2) 28.3 (CH_2) 32.4 (CH_2) 38.1 (CH_2) 46.9 (CH_2) 49.1 (CH) 56.8 (CH) 67.0 (CH_2) 109.3 (CH) 120.0 (CH) 124.1 (CH) 127.1 (CH) 127.5 (CH) 127.6 (CH) 128.0 (CH) 128.1 (CH) 128.5 (CH) 128.7 (CH) 129.2 (CH) 129.3 (CH) 130.5 (CH) 132.8 (C) 133.5 (CH) 136.1 (C) 136.4 (C) 140.0 (C) 145.2 (CH) 145.8 (C) 156.2 (CO) 171.2 (CO) ppm. HRMS (ES^+) $C_{36}H_{37}N_5O_5NaS$ (MNa^+) calcd. 674.2413; found 674.2393. $[\alpha]_D$ = –22 (*c* = 0.1, CH_2Cl_2).

Viability Assays. The effect of each final compound in the series on parasite growth was determined using the Alamar Blue cell viability assay. This assay was performed in triplicate according to Räz *et al*.[17] Briefly, *T. b. brucei* cells (strain MIT at 1.1) were seeded in 96-well plates at a density of 2×10^5 cells/mL in 100μL media in the presence of varying concentrations of predicted inhibitors (5 μM, 1 μM, 500 nm, 100 nM, 10 nM, 100 pM) or DMSO alone. A further 30 μL of media was added to each well. After 6 h, 15 μL of Alamar Blue (Invitrogen) was added to the cells and incubation continued so that the total incubation time was 24 h. Absorbances at 540 and 595 nm were measured using a SpectraMax M3 Microplate Reader (Molecular Devices), and EC_{50} values were calculated using the GraphPad Prism 5 software.

Acknowledgements

The Authors would like to thank The Health Research Board (A.K.), University College Dublin for financial support (W.D.) and the Irish Research Council for Science Engineering and Technology (IRCSET) for a postgraduate scholarship (N.A).

Supporting information: Copies of ^{1}H and ^{13}C NMR spectra are available electronically via:

References

1. For reviews including chemistry and biology of vinyl sulfones see: (a) N. S. Simpkins, *Tetrahedron*, 1990, **46**, 6951-6984; (b) P. L. Fuchs and T. F. Braish, *Chem. Rev*., 1986, **86**, 903-917; (c) A. El-Awa, M. N. Noshi, X. M. du Jardin and P. L. Fuchs, *Chem. Rev*., 2009, **109**, 2315-2349; (d) J. –E. Bäckvall, R. Chinchilla, C. Nájera and M. Yus, *Chem. Rev.*, 1998, **98**, 2291-2312; (e) D. C. Meadows and J. Gervay-Hague, *Med. Res. Rev*., 2006, **26**, 793-814; (f) M. M. M. Santos and R. Moreira, *Mini-Rev. Med. Chem.*, 2007, **7**, 1040-1050; (g) M. Verdoes, B. I. Florea, G. A. van der Marel and H. S. Overkleeft, *Eur. J. Org. Chem*., 2009, 3301-3313; (h) E. Dunny and P. Evans, *Curr. Bioact. Compd*., 2011, **7**, 218-236.

2. (a) A. R. Renslo and J. H. McKerrow, *Nat. Chem. Biol.*, 2006, **2**, 701–710; (b) A. H. Fairlamb, *Trends in Parasit.*, 2003, **19**, 488-494. For a recent monograph see: *Trypanosomatid Diseases: Molecular Routes to Drug Discovery*, T. Jäger, O. Koch and L. Flohé, Ed., Wiley-Blackwell, Weinheim, 2013.

3. E. Dunny, W. Doherty, P. Evans, J. P. G. Malthouse, D. Nolan and A. J. S. Knox, *J. Med. Chem.*, 2013, **56**, 6638–6650.

4. (a) J. C. Engel, P. S. Doyle, I. Hsieh and J. H. McKerrow, *J. Exp. Med.*, 1998, **188**, 725-734; (b) S. C. Barr, K. L. Warner, B. G. Kornreic, J. Piscitelli, A. Wolfe, L. Benet and J. H. McKerrow, *Antimicrob. Agents Chemother.*, 2005, **49**, 5160-5161; (c) P. S. Doyle, Y. M. Zhou, J. C. Engel and J. H. McKerrow, *Antimicrob. Agents Chemother.*, 2007, **51**, 3932-3939; (d) G. M. Lee, E. Balouch, D. H. Goetz, A. Lazic, J. H. McKerrow and C. S. Craik, *Biochemistry*, 2012, **51**, 10087-10098; (e) P. –Y. Yang, M. Wang, C. Y. He and S. Q. Yao, *Chem. Commun*., 2012, **48**, 835-837.

5. J. W. Choy, C. Bryant, C. M. Calvet, P. S. Doyle, S. S. Gunatilleke S. S. F. Leung, K. K. H. Ang, S. Chen, J. Gut, J. A. Oses–Prieto, J. B. Johnston, M. R. Arkin, A. L. Burlingame, J. Taunton, M. P. Jacobson J. M. McKerrow, L. M. Podust and A. R. Renslo, *Beilstein J. Org. Chem.*, 2013, **9**, 15–25.

6. (a) J. H. McKerrow, J. C. Engel and C. R. Caffrey. *Bioorg. Med. Chem.*, 1999, **7**, 639-644; (b) F. Lecaille, J. Kaleta and D. Brömme, *Chem. Rev.*, 2002, **102**, 4459-4488; (c) J. C. Powers, J. L. Asgian, Ö. D. Ekici and K. E. James, *Chem. Rev.*, 2002, **102**, 4639-4750; (d) R. Leung-Toung, Y. Yanqing Zhao, W. Wanren Li, T. F. Tam, K. Karimian and M. Spino, *Curr. Med. Chem.*, 2006, **13***,* 547-581; (e) D. Steverding, C. R. Caffrey and M. Sajid, *Mini-Rev. Med. Chem.*, 2006, **6**, 1025-1033; (f) R. Ettari, L. Tamborini, I. C. Angelo, N. Micale, A. Pinto, C. De Micheli and P. Conti, *J. Med. Chem.*, 2013, **56**, 5637–5658.

7. (a) I. D. Kerr, P. Wu, R. Marion–Tsukamaki, Z. B. Mackey and L. S. Brinen, *PLoS Negl. Trop. Dis.*, 2010, **4**, e701; (b) I. D. Kerr, J. H. Lee, C. J. Farady, R. Marion, M. Rickert, M. Sajid, K.C. Pandey, C. R. Caffrey, J. Legac, E. Hansell, J. H. McKerrow, C. S. Craik, P. J. Rosenthal and L. S. Brinen, *J. Biol. Chem.*, 2009, **284**, 25697-25703.

8. D. N. Gosalia, C. M. Salisbury, J. A. Ellman and S. L. Diamond, *Mol. Cell. Proteomics*, 2005, **4**, 626-636.

9. (a) S. Nahm and S. M. Weinreb, *Tetrahedron Lett.*, 1981, **22**, 3815-3818; (b) For a review concerning the use of Weinreb amides see: S. Balasubramaniam and I. Singh, Aidhen, *Synthesis*, 2008, 3707-3738.

10. For an example of the use of a Weinreb amide in the synthesis of dipeptide containing vinyl sulfones see: J. T. Palmer, D. Rasnick, J. L. Klaus and D. Brömme, *J. Med. Chem*., 1995, **38**, 3193-3196.

11. (a) E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2007, **9**, 3797–3800; (b) E. D. Goddard-Borger and R. V. Stick, *Org. Lett.*, 2011, **13**, 2514–2514; (c) N. Fischer, E. D. Goddard-Borger, R. Greiner, T. M. Klapötke, B.W. Skelton and J. Stierstorfer, *J. Org. Chem.*, 2012, **77**, 1760–1764.

12. For a recent example see: S. –J. Kim, H. –W. Rhee, H. –J. Park, H. –Y. Kim, H. –S. Kim and J.-I. Hong, *Bioorg. Med. Chem. Lett*., 2013, **23**, 2093-2097.

13. For selected examples of intramolecular vinyl sulfone *N*-conjugate addition see: J. J. Caldwell and D. Craig, *Angew. Chem. Int. Ed*., 2007, **46**, 2631-2634; (b) F. Iradier, R. Gómez Arrayás and J. C. Carretero, *Org. Lett*., 2001, **3**, 2957-2960; (c) J. de Vicente, R. Gómez Arrayás, J. Cañada and J. C. Carretero, *Synlett*, 2000, 53; (d) R. Gómez Arrayás and J. C. Carretero, *J. Org. Chem*., 1998, **63**, 2993-3005; (e) M. B. Berry, D. Craig, P. S. Jones and G. J. Rowlands, *Chem. Commun*., 1997, 2141-2142.

14. Azepanes **20** and **21** were analysed by ^{1}H NMR spectroscopy in CD_3OD over time and no interconversion was observed, either with or without the inclusion of Et_3N. For NOESY spectra of compounds **20**, **21** and **24**, see electronic supporting information.

15. (a) C. Godoy–Alcántar, A. K. Yatsimirsky and J. –M. Lehn, *J. Phys. Org. Chem.*, 2005, **18**, 979–985; (b) P. Kovaříček and J. –M. Lehn, *J. Am. Chem. Soc.*, 2012, **134**, 9446–9455; (c) M. Chan–Huot, A. Dos, R. Zander, S. Sharif, P. M. Tolstoy, S. Compton, E. Fogle, M. D. Toney, I. Shenderovich, G. S. Denisov and H. –H. Limbach, *J. Am. Chem. Soc.*, 2013, **135**, 18160–18175; (d) S. Ellis, D. S. Kalinowski, L. Leotta, M. L. Huang, P. Jelfs, V. Sintchenko, D. R. Richardson and J. A. Triccas, *Mol. Pharmacol*., 2014, **85**, 269-278.

16. F. Shi, J. P. Waldo, Y. Chen and R. C. Larock, *Org. Lett.*, 2008, **10**, 2409–2412.

17. B. Räz, M. Iten, Y. Grether-Bühler, R. Kaminsky and R. Brun, *Acta Trop.*, 1997, **68**, 139–147.

18. G. Wang and X. Q. Yao, *Org. Lett*., 2003, **5**, 4437–4440.

19. L. Fantetti, G. Adembri, A. Giotti, I. Masini and G. Roncucci, *Arzneimittel–Forschung*, 1999, **49**, 137–143.

20. H. Ye, R. Liu, D. Li, Y. Liu, H. Yuan, W. Guo, L. Zhou, X. Cao, H. Tian, J. Shen and P. G. Wang, *Org. Lett.*, 2013, **15**, 18-21.