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PAPER

Synthesis of conformationally constrained benzoylureas as BH3-mimetics†

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The design of small molecules that mimic the BH3 domain and bind to Bcl-2 proteins has emerged as a promising approach to discovering novel anti-cancer therapeutics. We reveal the design and synthesis of conformationally constrained benzoylurea scaffolds as conformational probes. Central to helix mimicry, the intramolecular hydrogen bond in the benzoylurea plays a key role in the pre-organisation of the acyclic substrates for cyclisation *via* ring closing metathesis, providing efficient access to the constrained mimetics.

Introduction

The regulation of programmed cell death (apoptosis) is a critical function in multicellular organisms. The balance between cell death and survival, if not closely regulated, has implications in a number of disease states, most notably cancer.[1,2] In the last decade research in the field has unravelled the biological intricacies of apoptosis and its role in cancer.[3,4] The proteins from the Bcl-2 family have been identified as key components in the apoptotic machinery.[5] These proteins can function as either pro-survival (Bcl-x_L, Mcl-1) or pro-apoptotic (Bim, Bax, Bak) and the interplay between the opposing factions controls the commitment to cell death. The BH3-only proteins are a subclass of the Bcl-2 family and play a key role in the commitment to apoptosis. In healthy cells, the Bcl-2 family keeps Bax/Bak in check, preventing apoptosis. Following apoptotic stimuli, the BH3-only proteins are released and bind to Bcl-2 and other pro-survival homologues. The binding event between BH3-only proteins and pro-survival proteins allows Bax and/or Bak to oligomerise in the mitochondrial outer membrane, releasing cytochrome *c*, which triggers caspase activation and results in cell death.[6]

Over-expression of Bcl-x_L has been implicated in many types of cancers.[7] Furthermore, chemoresistance can be attributed to impaired apoptosis resulting from over-expression of Bcl-x_L. Thus, a potential anticancer strategy is to neutralise Bcl-x_L by mimicking the BH3-only proteins with small molecules and thereby re-establishing the apoptotic process.[8] This approach has been greatly aided by the well-defined structural information obtained for the interaction between the BH3-only proteins and Bcl-x_L.[9–11] During the binding event, the BH3 domain of the BH3-only proteins, usually unstructured, adopts an α-helical conformation and inserts into a hydrophobic cleft on the surface of Bcl-x_L. Alanine scanning has implicated four hydrophobic residues along a continuous binding epitope as driving most of the binding energy.[9]

Our approach to BH3-mimetics entailed a computer-aided *de novo* design process utilising the key elements of the BH_3-domain/Bcl-x_L binding event. A benzoylurea was employed as an organic scaffold, mimicking the C_α–C_β bond vectors of the hydrophobic residues Phe, Ileu, and Leu.[12] The *in silico* designed benzoylurea **1** was found to have moderate affinity for Bcl-x_L, yet served as a valuable platform for medicinal chemistry optimisation. Subsequent efforts explored chemical space around the three hydrophobic groups resulting in **2**, a low micro molar inhibitor of Bcl-x_L (Fig. 1).[13]

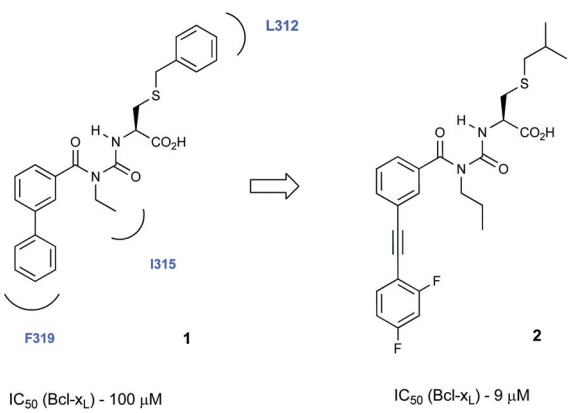

Fig. 1 The *de novo* designed BH3-mimetic based on a benzoylurea scaffold. Subsequent medicinal chemistry optimisation afforded **2** with low micromolar potency for Bcl-x_L.

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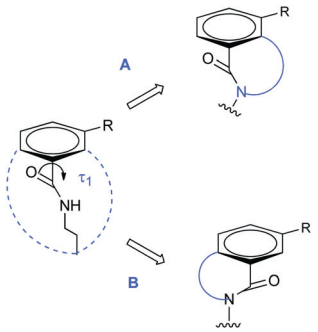


Fig. 2 Tethering the *N*-alkyl group to the aromatic ring, constraining the benzamide in both directions leading to two distinct sets of conformations A and B forms the strategy to probing the bioactive conformation.

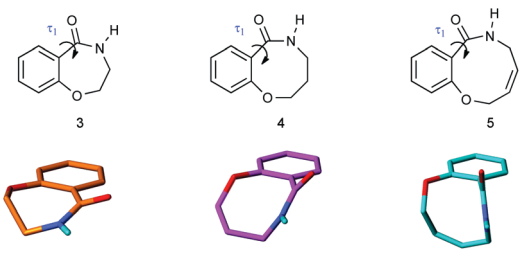


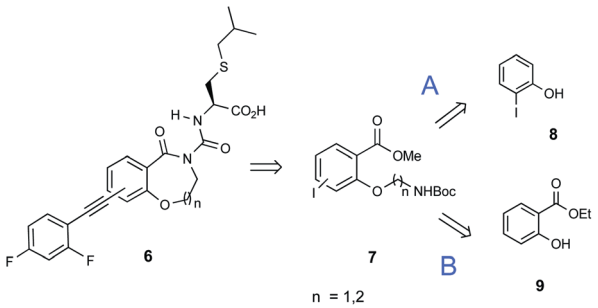
Fig. 3 Model lactam scaffolds and their energy minimised structures.

In this manuscript we focus on the design and synthesis of conformationally constrained analogues. Introducing conformational constraints in peptide mimetics through the formation of macrocycles such as lactams and cyclic peptides is a proven strategy to reduce unfavourable entropy loss upon binding and thus improve binding affinity,[14] the key being to emulate the receptor-bound conformation. However, in the absence of structural data, we had no such information on the correct conformation to induce in our ligand. Based on our earlier studies we were confident that the benzoylurea core would exist in the intramolecularly hydrogen-bonded conformation but we thought that the torsion angle τ_1 between the benzoyl phenyl ring and benzoyl carbonyl in these types of compounds could vary quite widely (Fig. 2).[15] We postulated that the receptor-bound conformation was not necessarily the intrinsically preferred conformation for this moiety in compound **2** and that an appropriate tether could restrict the molecule to its bioactive conformation, potentially improving binding affinity, as well as simultaneously informing us what the receptor-bound conformation was. Therefore, our strategy focused on introducing a tether between the *N*-alkyl moiety and the *ortho* position on the adjacent aromatic ring resulting in a lactam core. Tethering both *ortho* positions leads to constraining in two distinct conformations A and B which, could essentially control rotation and the torsion angle τ_1 of the benzamide bond (Fig. 2).

We reasoned that seven to nine membered lactam rings would comprise a suitable probe set and likely to explore torsion angles from relatively planar to significantly distorted. In order to seek support for this hypothesis, conformational analysis was carried out using the SYBYL8.0 software suite (Tripos Associates). Simplified lactams, representative of the type to be synthesised were used as model scaffolds for the analysis (Fig. 3). The

Table 1 Minimum energy conformations of the various lactams and the corresponding torsion angle

Entry	Lactam	Energy (kcal mol^{-1})	Torsion (°)
1	**3**	8.2	24
2	**4**	10.4	53
3	**5**	13.1	93
4	**5**	13.2	42
5	**5**	13.9	52
6	**5**	15.2	39



Scheme 1 Retrosynthesis of the constrained analogues using lactamisation chemistry.

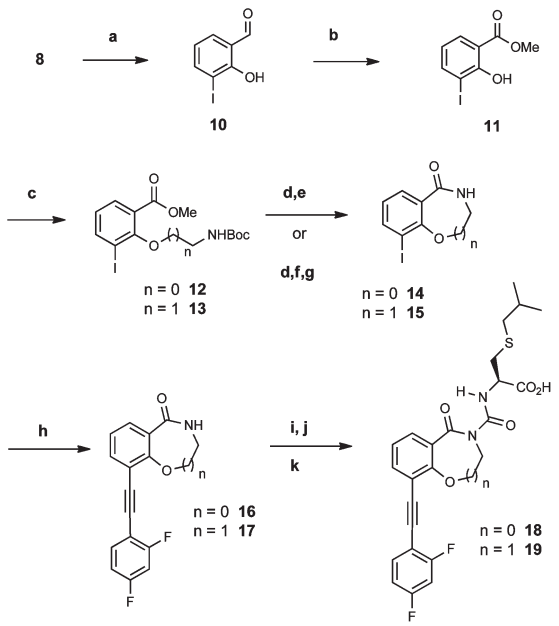
structures were generated *in silico*, energy minimised (Tripos force field, conjugate gradient method) and subjected to conformational searches (Table 1).

In the case of the seven and eight-membered lactams, the conformational search resulted in a global minimum conformer with respective τ_1 values of 24° and 53° (Table 1). However, the conformational complexity of the larger nine-membered lactam was exemplified by the identification of several conformers within 2 kcal mol^{-1} of the global minimum (Table 1). These computational calculations suggested that a range of τ_1 torsions would indeed be explored through the use of this set of constraints.

We envisaged that the synthesis of the seven and eight membered constrained analogues could be achieved through a lactamisation using intermediate **7**, which itself could be generated from 2-iodophenol **8** for conformer A or ethylsalicylate **9** for B (Scheme 1).

Synthesis of the seven and eight membered lactam analogues for conformer A began with an *ortho*-formylation of 2-iodophenol, affording **10** (Scheme 2).

The aldehyde was then converted directly to the ester **11** *via in situ* oxidation of the cyanohydrin, generated from NaCN and methanol.[16] Subsequent alkylation of the phenol with the appropriate *N*-Boc alkyl halide generated the lactamisation substrate. In the case of the seven-membered ring **14** cyclisation was facilitated following removal of the Boc protecting group from **12** and warming the resulting free amine to 60 °C in the presence of potassium carbonate. On the other hand, cyclisation of **13** to form the eight membered lactam **15** was not achievable under these conditions. Rather, hydrolysis of the ester and activation of the resulting carboxylic acid with carbodiimide facilitated cyclisation in a moderate 50% yield. Sonogashira cross coupling with 2,4-difluorophenylacetylene was employed to introduce the alkyne moiety in good yield for both **16** and **17**. Formation of the benzoylurea was achieved *via* formation of a carbamoyl



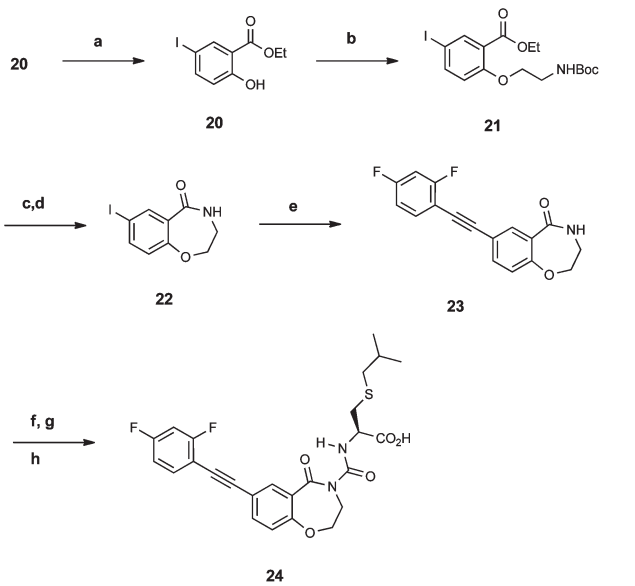
Scheme 2 Synthesis of seven and eight membered ring constrained analogues using lactamisation chemistry. Reagents and conditions: (a) $MgCl_2$, $(CH_2O)_n$, Et_3N, THF, 84%. (b) NaCN, MnO_2, MeOH, 77%. (c) $Br(CH_2)_nNHBoc$, Cs_2CO_3, DMF, 60 °C, 3 h. (d) TFA, DCM. (e) Na_2CO_3, *n*BuOH, 70 °C, 65%. (f) LiOH, MeOH–H_2O. (g) EDCI, HOBt, DIPEA, DCM, 12 h, 55%. (h) $Pd(OAc)_2$, PPh_3, CuI, piperidine, 2,4-difluorophenylacetylene, DMF, 70 °C, (i) TMSOTf, Et_3N, Et_2O, rt, 1 h. (j) 20% $COCl_2$ in toluene, rt, 2 h. (k) *N*,*N*-Bistrimethylsilylacetamide, propylene oxide, *S*-isobutyl cysteine, CH_3CN, rt, 1 h.

chloride intermediate resulting from activation of the amide group with trimethylsilyl triflate (TMSOTf) and subsequent reaction with phosgene.[17,18] In parallel, the carboxylic acid of the cysteine derivative[19] was TMS protected *in situ* by reaction with *N*,*N*-bistrimethylacetamide (BSA) followed by addition to the carbamoyl chloride. The TMS group is removed during the mild acidic (1N HCl) work up, revealing the constrained mimetics **18** and **19** (Scheme 2).

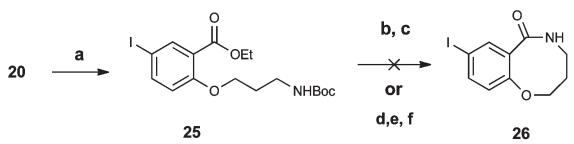
Introducing constraints on the other side of the molecule (conformer B) with a seven-membered ring began with the selective iodination *para* to the hydroxyl group of ethyl salicylate **9** with chloramine T in the presence of sodium iodide, yielding **20** (Scheme 3).[20] The phenol was efficiently alkylated with Boc-*N*-amino ethyl bromide, yielding the acyclic precursor **21**. Removal of the Boc group and warming to 70 °C in *n*-butanol facilitated cyclisation and formation of the lactam **22**. The alkyne was then introduced *via* Sonogashira coupling conditions furnishing **23**, followed by formation of the benzoylurea **24**.

We then attempted to replicate the lactamisation chemistry to prepare the eight-membered lactam **26** (Scheme 4). However, the combination of EDCI and HOBt with the carboxylic acid did not result in cyclisation as it did earlier to give **15**. We suspected that the inability to effect the intramolecular reaction was a result of the increase in conformational freedom of the longer *O*-alkyl chain, which could not be overcome by activation of the ester. As such, the eight-membered lactam appeared to be an ideal substrate for the RCM chemistry we have developed.

We have shown that intramolecular Grubb's ring closing metathesis (RCM) does not work for the preparation of lactams



Scheme 3 Synthesis of the seven-membered constraint. Reagents and conditions: (a) Chloramine T, NaI, DMF, 4 h, 82%. (b) $Br(CH_2)_2NHBoc$, Cs_2CO_3, DMF, 60 °C, 3 h, 65%. (c) TFA, DCM. (d) Na_2CO_3, *n*BuOH, 70 °C, 65%. (e) $Pd(OAc)_2$, PPh_3, CuI, piperidine, 2,4-difluorophenylacetylene, DMF, 70 °C, 65%. (f) TMSOTf, Et_3N, Et_2O, rt, 1 h. (g) 20% $COCl_2$ in toluene, rt, 2 h. (h) *N*,*N*-Bistrimethylsilylacetamide, propylene oxide, *S*-isobutyl cysteine, CH_3CN, rt, 1 h, 56% over three steps.



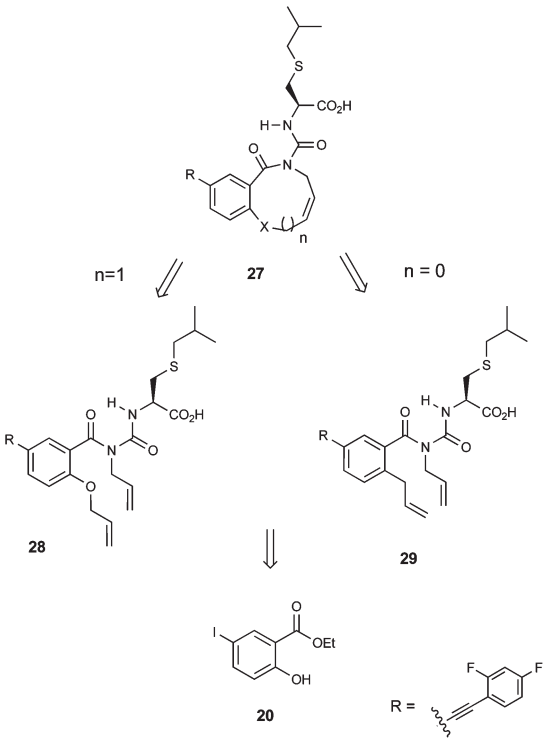
Scheme 4 Attempted synthesis of the eight-membered lactam *via* the lactamisation strategy. Reagents and conditions: (a) $Br(CH_2)_3NHBoc$, Cs_2CO_3, DMF, 60 °C, 3 h, 65%. (b) TFA, DCM. (c) Na_2CO_3, *n*BuOH, 70 °C. (d) TFA, DCM. (e) LiOH, MeOH, rt, 12 h. (f) EDCI, HOBT, DCM, rt, 12 h.

with an allylic alkene appended to a secondary amide, owing to the predominance of the *trans* amide bond.[21]

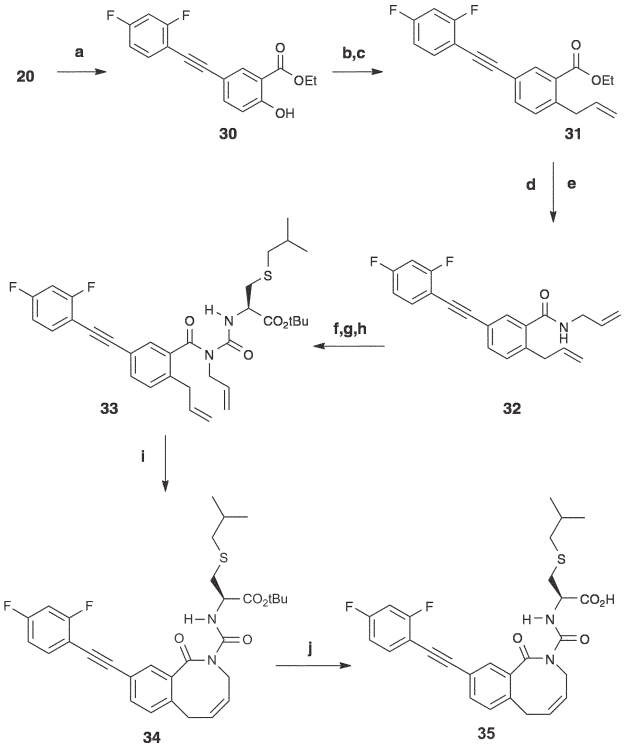
We have also demonstrated that the intramolecular hydrogen bond in benzoylureas pre-organises the amide group into a *cis* orientation, favouring the intramolecular cyclisation.[21] Thus, we decided to exploit the benzoylurea as a *cis* amide inducer by executing the RCM with substrates **28** and **29** in the penultimate step of the synthesis (Scheme 5).

As with previous syntheses, the phenol provides an easy point of attachment of an *O*-allylic moiety resulting in a precursor for the synthesis of the nine membered ring *via* RCM. In the case of the eight membered ring analogue however, we preferred a carbon–carbon linkage to introduce the shorter allyl group. For this approach, ethyl-4-iodo salicylate **20** could serve as a substrate to perform a C–C bond coupling following activation of the phenol.

Synthesis of the eight membered lactam began with a Sonogashira cross coupling between **20** and 2,4-difluorophenylacetylene to furnish the alkyne motif **30** (Scheme 6). After converting the phenol to the triflate, a Stille coupling using allyltributyltin



Scheme 5 Retrosynthesis of the constrained analogues employing RCM as the key step.



Scheme 6 Synthesis of the eight-membered constraint *via* RCM. Reagents and conditions: (a) $Pd(OAc)_2$, PPh_3, CuI, piperidine, 2,4-difluorophenylacetylene, DMF, 70 °C, 65%. (b) Tf_2O, DMAP, collidine, DCM, −15 °C to rt, 4 h, 89%. (c) $Pd(PPh_3)_4$, allyltributyltin, DMF, 80 °C, 78%. (d) LiOH, MeOH, rt, 12 h. (e) EDCI, HOBT, allyl amine, DCM, rt, 12 h, 67%. (f) TMSOTf, Et_3N, Et_2O, rt, 1 h. (g) 20% $COCl_2$ in toluene, rt, 2 h. (h) iso-Butylcysteine hydrochloride *tert*-butyl ester, DIPEA, CH_3CN, rt, 55%. (i) Grubbs-II, DCM, 40 °C, 1 h, 70%. (j) TFA, DCM, rt, 4 h, 94%.

resulted in the formation of **31**. The second terminal alkene was then introduced after hydrolysis of the ester and EDCI promoted coupling of the acid with allyl amine affording **32**. Introducing the benzoylurea formed the key metathesis substrate **33**.[22]

The 'closed' conformation of the benzoylurea was evidenced by the de-shielded N–H resonance, confirming the presence of the intramolecular hydrogen bond (9.8 ppm).[15] With this 'closed' conformation stabilised, we were pleased to observe a rapid RCM of the terminal alkenes in **33** to **34**. Treatment with TFA removed the ester and afforded the constrained mimetic **35**. Similarly, the nine-membered lactam could be accessed by introducing the terminal alkenes **37** in three steps from the phenol **30** (Scheme 7). Formation of the benzoylurea **38** facilitated efficient cyclisation to the nine-membered lactam **39**. Acidolysis revealed the carboxylic acid, affording the nine-membered constrained mimetic **40**.

The ability of the constrained analogues to bind to Bcl-x_L was evaluated using AlphaScreen assay technology.[23,24] The AlphaScreen is a bead-based competition assay, whereby biomolecules are linked to donor and acceptor beads. Interaction of the two proteins brings the beads into proximity, generating a fluorescence signal. Inhibition of the protein–protein interaction results in a decrease in the fluorescence signal and can be quantified as an IC_{50}. In this particular assay, the donor bead is coated with streptavidin and conjugated with biotinylated Bim peptide. The acceptor bead is coated with an anti-glutathione S-transferase (GST) antibody conjugated to GST tagged Bcl-x_L.

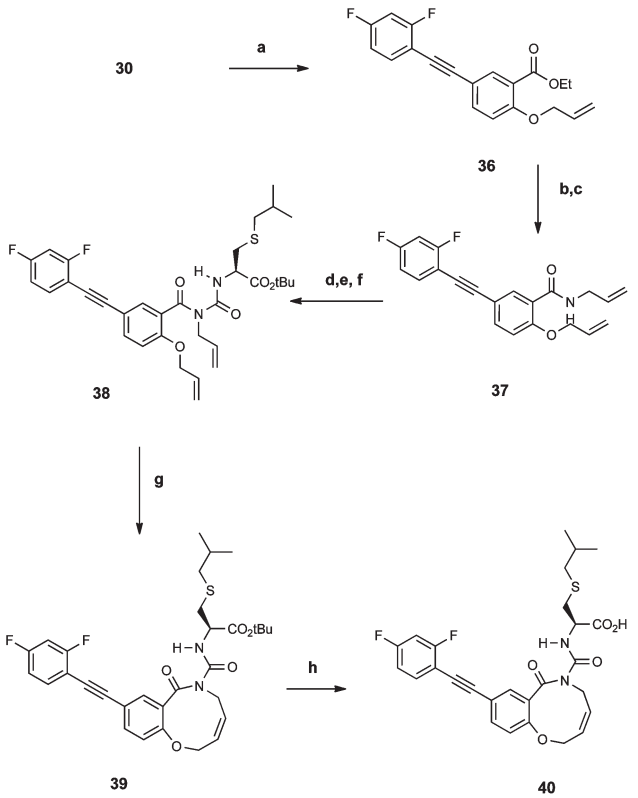
As shown in Table 2, binding affinity seems to increase with ring size. Constraining with a seven membered lactam essentially rendered the molecule inactive, while the nine membered lactam **40** displayed a reasonably potent IC_{50} of 57 μM. This implies that a bioactive conformation involving a τ_1 torsion angle significantly distorted from planarity is preferred. However, overall the introduction of conformational constraints did not result in the expected increase in IC_{50} compared with **2**. There are several possible explanations for this result. In particular, we know from SAR studies that loss of the propyl group abrogates binding affinity.[13] In our constrained analogues, the alkyl tether may not be able to occupy the same hydrophobic pocket. Thus, our future set of targets will focus on analogues that incorporate both the constraints and an equivalent of the propyl chain as more potent Bcl-x_L inhibitor.

While the benzoylurea is an effective scaffold for the design of helical mimetics, we have shown that the 'closed' conformation of this motif can have an important application in synthetic chemistry: our work demonstrates its potential for the preparation of macrocyclic lactams *via* RCM.

Experimental section

Chemistry

All non-aqueous reactions were carried out in oven-dried glassware under an atmosphere of nitrogen, unless otherwise



Scheme 7 Synthesis of the nine-membered lactam *via* RCM. Reagents and conditions: (a) Allyl bromide, Cs_2CO_3, DMF, 60 °C, 4 h, 88%. (b) LiOH, MeOH, rt, 12 h. (c) EDCI, HOBT, allyl amine, DCM, rt, 12 h, 48%. (d) TMSOTf, Et_3N, Et_2O, rt, 1 h. (e) 20% $COCl_2$ in toluene, rt, 2 h. (f) iso-Butylcysteine hydrochloride *tert*-butyl ester, DIPEA, CH_3CN, rt, 65%. (g) Grubbs-II, DCM, 40 °C, 1 h, 78%. (h) TFA, DCM, rt, 4 h, 90%.

Table 2 Alpha screen IC_{50}'s for the constrained BH3-mimetics

Entry	Ring size (conformer)	Compound	IC_{50} (μM)
1	—	**2**	9
2	7(A)	**18**	>100
3	8(A)	**19**	74
4	7(B)	**24**	100
5	8(B)	**35**	66
6	9(B)	**40**	57

specified. DCM, THF, DMF and diethyl ether were dried on an M-Braun Solvent Purification system. Anhydrous acetonitrile (99.8%) was purchased from Sigma-Aldrich and used as supplied. Triethylamine was distilled over calcium hydride and stored over molecular sieves. Petroleum ether describes a mixture of hexanes in the bp range 40–60 °C. Analytical thin-layer chromatography was performed on Merck silica gel $60F_{254}$ aluminium-backed plates and were visualised by fluorescence quenching under UV light and/or a permanganate stain consisting of $KMNO_4$ (3.0 g), K_2CO_3 (20 g) and 5% w/v aqueous NaOH (5 mL) in H_2O (300 mL). Flash column chromatography was performed with silica gel 60 (particle size 0.040–0.063 mm). All NMR spectra were recorded on a Bruker Avance DRX 300 with solvents indicated (^{1}H NMR at 300 MHz; ^{13}C at 75 MHz). Chemical shifts are reported in ppm on the δ scale, referenced to the appropriate solvent peak. Infrared spectra were recorded on a Bruker Tensor27 FTIR spectrometer and absorptions are given in wavenumbers (cm^{-1}). Oils were analysed using sodium chloride plates. Solids were analysed using a diffuse resistance accessory. Mass spectrometry was performed on a Finigan LCQ Advantage MAX. High-resolution mass spectra were measured at the Australian National University on a Bruker Apex 3 HR FTICR ESI mass spectrometer. Elemental analyses were carried out for carbon, nitrogen, hydrogen and sulfur at the university of Otago microanalytical laboratory. Preparative HPLC purification was performed using a Waters 2795 Alliance HT with a Phenomenex Luna 5 μ C18 column (100 Å, flow-rate 4.0 ml min^{-1}), 0–50% $CH_3CN : H_2O$ gradient containing 0.1% formic acid.

For all minimizations and force field calculations, Tripos force field was used with Gasteiger–Huckel charges as implemented in SYBYL8.0 (Tripos Associates; http://www.tripos.com). Default settings were used for the minimization process, including the use of a distance dependant dielectric of 1 using a non-bonded cut-off of 8 Å.

Experimental procedures and full characterisation data for compounds **10–17**, **20–23**, **25** and **30–32** are included in the ESI.†

General procedure A: carbamoyl chloride synthesis

To a stirred suspension of the amide in anhydrous diethyl ether (2 mL per 0.5 mmol) was added anhydrous Et_3N (1.1 eq), followed by TMSOTf (1.1 eq). The reaction was stirred under N_2 at room temperature for 1 h. After that time, the solution was cooled to 0 °C and then treated with a solution of phosgene in toluene (20% in toluene, 100 μL per 0.1 mmol). The mixture was warmed to room temperature and stirred for 1 h. After this time, the reaction flask was connected to a vacuum line and concentrated, leaving the carbamoyl chloride as a viscous oil.

General procedure B: benzoylurea synthesis

To a suspension of the amino acid hydrochloride in acetonitrile (4 mL per mmol) was added propylene oxide (2 mL per mmol) and *N*,*O*-bistrimethylacetamide (1.5 eq). The reaction mixture was stirred at room temperature under N_2 for 1 h. The carbamoyl chloride, obtained according to general procedure C, was dissolved in acetonitrile (1 mL per 0.5 mmol). At 0 °C, this solution was added to the *in situ* protected amino acid. The reaction was warmed to room temperature and allowed to stir for a further 15 min. After this time the mixture was diluted with EtOAc and poured onto 2 N HCl (5 mL for 1 mmol carbamoyl chloride). The aqueous phase was extracted three times with EtOAc and the combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The residues were purified *via* column chromatography, eluting with CH_2Cl_2–MeOH–AcOH.

General procedure C: ring closing metathesis

In an oven-dried 2-neck flask, the diene was dissolved in anhydrous DCM (331 mL per mmol) and treated with Grubb's

second-generation catalyst (5 mol%) under an atmosphere of N_2. The reaction mixture was refluxed for 2 h, after which time it was concentrated. The dark-brown oil was purified *via* column chromatography eluting with EtOAc–pet. ether.

(*R*)-2-(9-((2,4-Difluorophenyl)ethynyl)-5-oxo-2,3,4,5-tetrahydrobenzo[*f*][1,4]oxazepine-4-carboxamido)-3-(isobutylthio)propanoic acid (18). The benzoylurea **18** was synthesised from the amide **16** (0.080 g, 0.26 mmol) according to general procedures A and B. Flash column chromatography, eluting with DCM–MeOH (98 : 2), then DCM–MeOH–AcOH (98 : 1 : 1), afforded a viscous yellow oil (0.064 g, 50%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.83 (d, J = 6.8 Hz, 1H, NH), 7.67–7.47 (m, 2H), 7.43 (ddd, J = 16.1, 6.3 and 6.3 Hz, 1H, CH), 7.20–7.11 (m, 1H), 6.84–6.77 (m, 2H), 4.71–4.69 (m, 1H), 4.45–4.42 (m, 2H), 4.14–4.01 (m, 2H), 3.02 (dd, J = 5.1 and 3.6 Hz, 2H, CH_2), 2.43 (d, J = 6.8 Hz, 2H, CH_2), 1.75 (m, 1H), 0.91 (d, J = 6.6 Hz, 6H, 2 × CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.6 (C), 164.7 (C), 161.3 (C), 153.7 (C), 153.5 (C), 137.5 (CH), 134.6 (CH), 134.2 (C), 130.9 (CH), 129.1 (CH), 124.2 (C), 117.2 (C), 111.7 (CH), 104.8 (C), 104.4 (CH), 90.1 (C), 85.6 (C), 73.2 (CH_2), 54.6 (CH), 42.0 (CH_2), 41.7 (CH), 32.7 (CH_2), 28.7 (CH), 21.9 (2 × CH_3); MS (ES^+) m/z 503 (M + H); IR (ATR cm^{-1}) 3224, 2962, 2337, 1697, 1504, 1342, 1095; HRMS (ES^-) Calculated for $C_{25}H_{23}N_2O_5S$ (M − H): 501.1296; found 501.1296.

(*R*)-2-(10-((2,4-Difluorophenyl)ethynyl)-6-oxo-3,4,5,6-tetrahydro-2*H*-benzo[*b*][1,5]oxazocine-5-carboxamido)-3-(isobutylthio)propanoic (19). The benzoylurea **19** was prepared from the amide **17** (0.030 g, 0.10 mmol) according to general procedures A and B. Flash column chromatography, eluting with DCM–MeOH (98 : 2), then DCM–MeOH–AcOH (98 : 1 : 1), afforded a viscous yellow oil (0.026 g, 53%) ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.1 (d, J = 6.7 Hz, 1H, NH), 7.65 (dd, J = 7.6 and 1.7 Hz, 1H, CH), 7.54–7.47 (m, 2H), 7.17–7.12 (m, 1H), 6.93–6.85 (m, 2H), 4.78 (dd, J = 12.4 and 6.0 Hz, 1H, CH), 4.57–4.11 (m, 2H), 3.38–3.29 (m, 2H), 3.11–3.02 (m, 2H), 2.51 (d, J = 6.5 Hz, 2H, CH_2), 2.28 (br. s, 1H, CH), 1.98 (br. s, 1H, CH), 1.82 (m, 1H, CH), 0.96 (d, J = 6.6, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2 (C), 172.1 (C), 164.1 (C), 161.3 (C), 157.9 (C), 154.5 (CH), 136.4 (C), 134.3 (CH), 134.1 (CH), 130.6 (CH), 124.8 (C), 123.8 (CH), 117.4 (C), 115.2 (C), 111.8 (CH), 104.3 (CH), 91.3 (C), 84.4 (C), 73.5 (CH_2), 53.8 (CH), 43.5 (CH_2), 42.0 (CH_2), 34.2 (CH_2), 28.6 (CH), 21.9 (2 × CH_3); IR (ATR cm^{-1}) 3026, 2962, 2358, 1734, 1576, 1436; MS (ES^+) m/z 517 (M + H); HRMS (ES^+) Calculated for $C_{26}H_{27}F_2N_2O_5S$ (M + H): 517.1609; found 517.1609.

(*R*)-2-(7-((2,4-Difluorophenyl)ethynyl)-5-oxo-2,3,4,5-tetrahydrobenzo[*f*][1,4]oxazepine-4-carboxamido)-3-(isobutylthio)propanoic acid (24). The benzoylurea **24** was synthesised from the amide **16** (0.060 g, 0.23 mmol) according to general procedures A and B. Flash column chromatography, eluting with DCM–MeOH (98 : 2), then DCM–MeOH–AcOH (98 : 1 : 1), afforded the benzoylurea as a clear oil (0.056 g, 56%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.92 (d, J = 7.0 Hz, 1H, CH), 7.96 (d, J = 2.0 Hz, 1H, CH), 7.65 (dd, J = 8.3 and 2.2 Hz, 1H, CH), 7.52–7.26 (m, 1H), 7.06–7.03 (m, 1H), 6.92–6.84 (m, 2H), 4.82–4.76 (m, 1H), 4.44–4.41 (m, 2H), 4.15–4.10 (m, 2H), 3.11–3.03 (m, 2H), 2.50 (d, J = 6.8 Hz, 2H, CH_2), 1.82 (sep, J = 6.8 Hz, 1H), 0.99 (d, J = 6.6 Hz, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.2 (C), 170.6 (C), 164.3 (C), 161.3 (C), 153.9 (C), 153.8 (C), 137.3 (CH), 134.5 (CH), 134.2 (C), 132.1 (CH), 127.8 (CH), 122.4 (C), 118.9 (C), 111.6 (CH), 104.3 (CH), 92.3 (C), 82.4 (C), 73.5 (CH_2), 53.8 (CH), 41.9 (CH_2), 41.6 (CH_2), 34.2 (CH_2), 29.6 (CH), 21.9 (CH_3), 21.8 (CH_3); IR (ATR cm^{-1}) 3752, 3071, 2962, 2358, 1734, 1684, 1517, 1096; MS (ES^+) m/z 503 (M + H); HRMS (ES^-) Calculated for $C_{25}H_{23}F_2N_2O_5S$ (M − H): 501.1296; found 501.1296.

(*R*)-*tert*-Butyl-2-(3-allyl-3-(2-allyl-5-((2,4-difluorophenyl)ethynyl)-benzoyl)ureido)-3-(isobutylthio)propanoate (33). According to general procedure A, **32** (0.147 g, 0.44 mmol) was converted to the carbamoyl chloride. The carbamoyl chloride was then dissolved in CH_3CN (4 mL). At 0 °C iso-butylcysteine hydrochloride *tert*-butyl ester (0.154 g, 0.57 mmol) and DIPEA (0.382 mL, 2.2 mol) were added and the solution warmed to room temperature over 15 min. The mixture was quenched with 1 N HCl (10 mL) and extracted with EtOAc (2 × 15 mL). The combined organics were washed with sat. $NaHCO_3$ (10 mL), water (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification *via* column chromatography, eluting with EtOAc–pet. ether (20 : 80) afforded **33** as a clear oil (0.143 g, 55%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.76 (d, J = 7.3 Hz, 1H, NH), 7.54–7.25 (m, 4H), 6.91–6.84 (m, 2H), 5.93–5.72 (m, 2H), 5.16–4.89 (m, 4H), 4.68 (dd, J = 6.9 and 5.8 Hz, 1H, CH), 4.48–4.11 (br. m, 2H), 3.39 (d, J = 6.8 Hz, 2H, CH_2), 3.03 (dd, J = 8.7 and 4.9 Hz, 2H, CH_2), 2.48 (dd, J = 7.1 and 1.5 Hz, 2H, CH_2), 1.82 (sept, J = 6.7 Hz, 1H, CH), 1.51 (s, 9H, 3 × CH_3), 0.98 (d, J = 6.3 Hz, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 173.5 (C), 169.3 (C), 164.6 (C), 161.2 (C), 153.7 (C), 136.6 (C), 135.9 (C), 135.1 (CH), 134.3 (CH), 133.1 (CH), 132.8 (CH), 130.0 (CH), 128.5 (CH), 120.9 (C), 117.6 (CH_2), 117.2 (CH_2), 111.7 (CH), 107.9 (C), 104.3 (CH), 92.8 (C), 82.5 (C), 82.3 (C), 54.6 (CH), 48.9 (CH_2), 42.1 (CH_2), 37.4 (CH_2), 34.8 (CH_2), 28.5 (CH), 27.9 (3 × CH_3), 21.9 (2 × CH_3); MS (ES^+) m/z 597 (M + H); IR (ATR cm^{-1}) 3221, 3071, 2958, 2138, 1706, 1074; HRMS (ES^+) Calculated for $C_{33}H_{39}F_2N_2O_4S$ (M + H): 597.2599; found 597.2599.

(*R*,*Z*)-*tert*-Butyl 2-(9-((2,4-difluorophenyl)ethynyl)-1-oxo-1,2,3,6-tetrahydrobenzo[*c*]azocine-2-carboxamido)-3-(isobutylthio)-propanoate (34). The cyclic alkene was prepared from the diene **33** (0.122 g, 0.21 mmol) according to general procedure C. Flash column chromatography, eluting with EtOAc–pet. ether (30 : 70), afforded **34** as a light grey solid (0.084 g, 70%); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.82 (d, J = 6.6 Hz, 1H, NH), 7.61–7.57 (m, 2H), 7.52–7.44 (m. 2H), 7.15–7.13 (m, 1H), 6.89–6.83 (m, 1H), 5.95–5.84 (m, 2H), 4.78–4.76 (m, 1H), 4.46–4.40 (m, 1H), 3.77–3.58 (m, 2H), 3.30 (br. s, 1H, CH), 3.08–3.02 (m, 2H), 2.48 (d, J = 5.9 Hz, 2H, CH_2), 1.78 (m, 1H), 0.97 (d, J = 6.0 Hz, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.1 (C), 169.8 (C), 166.8 (C), 164.5 (C), 155.6 (C), 138.6 (C), 136.8 (CH), 134.4 (CH), 134.3 (CH), 133.5 (C), 129.9 (CH), 129.1 (CH), 128.9 (CH), 122.1 (C), 111.6 (CH), 107.2 (C), 104.3 (CH), 92.6 (C), 83.1 (C), 54.6 (CH), 43.2 (CH_2), 42.8 (CH_2), 34.9 (CH_2), 32.7 (CH_2), 28.7 (CH), 21.9 (2 × CH_3); IR (ATR cm^{-1}) 3270, 2957, 2873, 1698, 1508, 1144; MS (ES^+)

m/*z*, 513 (M + H); HRMS (ES^+) Calculated for $C_{27}H_{25}F_2N_2O_4S$ (M − H): 511.1503; found 511.1503.

(*R*,*Z*)-2-(9-((2,4-Difluorophenyl)ethynyl)-1-oxo-1,2,3,6-tetrahydrobenzo[*c*]azocine-2-carboxamido)-3-(isobutylthio)propanoic acid (35). The ester **34** (0.049 g, 0.08 mmol) was dissolved in DCM (1 mL). TFA (1 mL) was added and the resulting brown solution stirred at room temperature for 2 h. The solution was diluted with toluene (2 mL) and concentrated. This process was repeated 3 times, affording **35** as a light brown residue (0.042 g, 94%); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.82 (d, J = 6.6 Hz, 1H, NH), 7.61–7.57 (m, 2H), 7.52–7.44 (m. 2H), 7.15–7.13 (m, 1H), 6.89–6.83 (m, 1H), 5.95–5.84 (m, 2H), 4.78–4.76 (m, 1H), 4.46–4.40 (m, 1H), 3.77–3.58 (m, 2H), 3.30 (br. s, 1H, CH), 3.08–3.02 (m, 2H), 2.48 (d, J = 5.9 Hz, 2H, CH_2), 1.78 (m, 1H), 0.97 (d, J = 6.0 Hz, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.1 (C), 169.8 (C), 166.8 (C), 164.5 (C), 155.6 (C), 138.6 (C), 136.8 (CH), 134.4 (CH), 134.3 (CH), 133.5 (C), 129.9 (CH), 129.1 (CH), 128.9 (CH), 122.1 (C), 111.6 (CH), 107.2 (C), 104.3 (CH), 92.6 (C), 83.1 (C), 54.6 (CH), 43.2 (CH_2), 42.8 (CH_2), 34.9 (CH_2), 32.7 (CH_2), 28.7 (CH), 21.9 (2 × CH_3); IR (ATR cm^{-1}) 3244, 2337, 1705, 1612, 1149, 1033; MS (ES^+) *m*/*z*, 513 (M + H); HRMS (ES^+) Calculated for $C_{27}H_{25}F_2N_2O_4S$ (M − H): 511.1503; found 511.1503.

(*R*)-*tert*-Butyl 2-(3-allyl-3-(2-(allyloxy)-5-((2,4-difluorophenyl)-ethynyl)benzoyl)ureido)-3-(isobutylthio)propanoate (38). According to general procedure A, **37** (0.270 g, 0.76 mmol) was converted to the corresponding carbamoyl chloride and then dissolved in CH_3CN (1.5 mL). At 0 °C iso-butylcysteine hydrochloride *tert*-butyl ester (0.266 g, 1.0 mmol) and DIPEA (0.661 mL, 4.0 mmol) were added and the solution warmed to room temperature over 15 min. The mixture was quenched with 1 N HCl (10 mL) and extracted with EtOAc (2 × 15 mL). The combined organics were washed with sat. $NaHCO_3$ (10 mL), water (10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification *via* flash column chromatography, eluting with EtOAc–hexane (0 : 100 to 10 : 90) afforded **38** as a clear oil (0.304 g, 65%); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.73 (d, J = 5.5 Hz, 1H, NH), 7.53–7.40 (m, 3H), 6.90–6.81 (m, 3H), 6.00–5.91 (m, 1H), 5.77–5.67 (m, 1H), 5.39–5.32 (m, 2H), 5.10–4.87 (m, 2H), 4.66–4.58 (m, 3H), 4.52–4.36 (br. m, 1H), 4.05–3.88 (br. m, 1H), 3.02–2.96 (m, 2H), 2.47 (d, J = 6.6 Hz, 2H, CH_2), 1.78 (sept, J = 6.6 Hz, 1H, CH), 1.46 (s, 9H, 3 × CH_3), 0.97 (d, J = 6.6 Hz, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.6 (C), 169.4 (C), 164.4 (C), 161.1 (C), 153.8 (C), 153.7 (C), 134.4 (CH), 134.2 (CH), 133.5 (CH), 131.9 (CH), 130.8 (CH), 126.6 (C), 118.0 (CH_2), 116.6 (CH_2), 115.5 (C), 112.4 (CH), 111.6 (CH), 108.1 (C), 104.3 (CH), 92.8 (C), 82.4 (C), 81.5 (C), 69.3 (CH_2), 54.7 (CH), 48.3 (CH_2), 42.1 (CH_2), 34.8 (CH_2), 28.6 (CH), 27.9 (3 × CH_3), 21.9 (2 × CH_3); IR (ATR cm^{-1}) 3283, 3077, 2850, 1733, 1631, 1541, 1010; MS (ES^+) *m*/*z* 613 (M + H); HRMS (ES^+) Calculated for $C_{33}H_{39}F_2N_2O_5S$ (M + H): 613.2548; found 613.2548.

(*R*,*Z*)-*tert*-Butyl 2-(9-((2,4-difluorophenyl)ethynyl)-7-oxo-2,5,6,7-tetrahydrobenzo[*b*][1,5]oxazonine-6-carboxamido)-3-(isobutylthio)-propanoate (39). The cyclic alkene **39** was prepared from the diene **38** (0.153 g, 0.25 mmol) according to general procedure C. Flash column chromatography, eluting with EtOAc–pet. ether (30 : 70), afforded a brown oil (0.102 g, 70%); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.86 (d, J = 6.9 Hz, 1H, NH), 7.67–7.62 (m, 2H), 7.51–7.43 (m, 1H), 7.01 (dd, J = 8.3 and 0. 5 Hz, 1H, CH), 6.91–6.83 (m, 2H), 6.25–6.16 (m, 1H), 5.98–5.92 (m, 1H), 4.83 (br. s, 2H, CH_2), 4.69–4.63 (m, 1H), 4.03–3.98 (m, 2H), 3.04–3.00 (m, 2H), 2.47 (d, J = 6.8 Hz, 2H, CH_2), 1.77 (sept, J = 6.7 Hz, 1H, CH), 1.51 (s, 9H, 3 × CH_3), 0.97 (d, J = 6.6 Hz, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 171.2 (C), 169.4 (C), 164.6 (C), 161.2 (C), 156.6 (C), 154.1 (C), 135.7 (CH), 134.2 (C), 132.5 (CH), 132.0 (CH), 130.1 (CH), 128.6 (C), 118.3 (CH), 118.2 (CH), 111.6 (CH), 107.9 (C), 92.5 (C), 82.4 (C), 82.1 (C), 71.6 (CH_2), 54.7 (CH_2), 46.1 (CH_2), 42.2 (CH_2), 34.8 (CH_2), 28.6 (CH), 27.9 (CH_3), 21.9 (CH_3), 21.8 (CH_3); IR (ATR cm^{-1}) 2958, 2330, 1719, 1578, 1368, 1164, 752; MS (ES^+) *m*/*z* 585 (M + H); HRMS (ES^+) Calculated for $C_{31}H_{35}F_2N_2O_5S$ (M + H): 585.2235; found 585.2235.

(*R*,*Z*)-2-(9-((2,4-Difluorophenyl)ethynyl)-7-oxo-2,5,6,7-tetrahydrobenzo[*b*][1,5]oxazonine-6-carboxamido)-3-(isobutylthio)-propanoic acid (40). 39 (0.061 g, 0.10 mmol) was dissolved in DCM (0.100 mL) and treated with TFA (0.40 mmol, 0.032 mL). After 2 h, the solution was diluted with toluene (2 mL) and concentrated. This process was repeated three times, affording **40** as a light brown residue (0.052 g, 94%); ^1H NMR ($CDCl_3$, 300 MHz) δ 9.93 (1H, d, J = 6.5 Hz, NH), 7.68–7.64 (m, 2H), 7.51–7.43 (m, 1H), 7.02 (d, J = 8.7 Hz, 1H, CH), 6.91–6.83 (m, 2H), 6.22–6.16 (m, 1H), 5.99–5.93 (m, 1H), 4.84–4.73 (m, 3H), 4.10–4.00 (m, 2H), 3.10 (dd, J = 3.6 and 2.5 Hz, 2H, CH_2), 2.40 (d, J = 6.3 Hz, 2H, CH_2), 1.81 (sept, J = 6.7 Hz, 1H, CH), 0.98 (d, J = 6.6 Hz, 6H, 2 × CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 174.5 (C), 171.5 (C), 164.6 (C), 161.2 (C), 156.6 (C), 154.1 (C), 135.8 (CH), 134.3 (C), 134.1 (CH), 132.6 (CH), 132.3 (CH), 129.8 (CH), 128.3 (C), 118.2 (CH), 111.6 (CH), 107.8 (C), 104.5 (CH), 92.6 (C), 82.2 (C), 71.7 (CH_2), 54.7 (CH), 46.3 (CH_2), 42.0 (CH_2), 34.1 (CH_2), 28.6 (CH), 21.9 (2 × CH_3); IR (ATR cm^{-1}) 3272, 3029, 2920, 1695, 1509, 1371, 1107; MS (ES^+) *m*/*z* 529 (M + H); IR (ATR cm^{-1}) 3081, 2978, 1780, 1654, 1522, 1171; HRMS (ES^-) Calculated for $C_{27}H_{25}F_2N_2O_5S$ (M − H): 527.1452; found 527.1452.

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