Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 15 (2005) 1327–1331
Bioorganic & Medicinal Chemistry Letters

cis-6-Oxo-hexahydro-2-oxa-1,4-diazapentalene and *cis*-6-oxo-hexahydropyrrolo[3,2-*c*]pyrazole based scaffolds: design rationale, synthesis and cysteinyl proteinase inhibition

Yikang Wang, Alex Benn,† Nick Flinn, Tracy Monk, Manoj Ramjee, John Watts and Martin Quibell*

Amura Therapeutics Limited, Incenta House, Horizon Park, Barton Road, Comberton, Cambridge CB3 7AJ, UK

Received 11 October 2004; revised 7 January 2005; accepted 12 January 2005

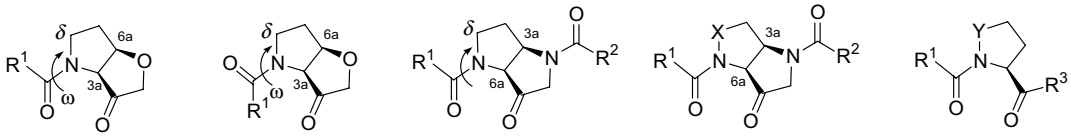
Abstract—The 5,5-bicycles *cis*-6-oxo-hexahydro-2-oxa-1,4-diazapentalene **3** and *cis*-6-oxo-hexahydropyrrolo[3,2-*c*]pyrazole **4** were designed as rotationally restricted templates towards the preparation of inhibitors of CAC1 cysteinyl proteinases. The design strategy was exemplified through the solution and solid phase preparation of potent inhibitors of human cathepsin K and may potentially be applied to inhibitors of other CAC1 proteinases.

Inhibitors of the CAC1 class of proteolytic enzyme[1] have the potential to treat a wide range of indications such as osteoporosis, multiple sclerosis, rheumatoid and osteoarthritis, atherosclerosis and cancer as well as parasitic infections such as malaria and Chagas disease.[2–4] Consequently, a significant resource is currently devoted within the pharmaceutical industry towards the development of CAC1 proteinase inhibitors,[5] which has recently provided the first clinical candidates with cathepsin K as the target and osteoporosis as the main therapeutic indication. In the search for new protease inhibitors, we have described the design, synthesis and inhibition kinetics for bicyclic peptidomimetics **1**[6] and **2**[5] that are constrained scaffolds providing potent and selective inhibitors of CAC1 cysteinyl proteinases. A key design feature of **1** and **2** was stabilisation of the otherwise chirally labile position situated α to the ketone,[7] by exploiting the kinetic and thermodynamic stability of a *cis*-fused 5,5-bicycle. We have now extended the design process and herein report the rationale, synthesis and inhibition kinetics for the new heterobicycles *cis*-6-oxo-hexahydro-2-oxa-1,4-diazapentalene **3** and *cis*-6-oxo-hexahydropyrrolo[3,2-*c*]pyrazole **4**.

We have previously reported our molecular modelling studies based on *N*-acylated leucine analogues of scaffolds **1** and **2** with cathepsin K.[5,6] These studies predicted that the leucine carbonyl could form a conserved hydrogen bond with the proteinase backbone N–H of glycine66. However, within the predicted bioactive conformation of the *si* tetrahedral intermediate, [6] this required the carbonyl group to occupy approximately the same plane as the 5,5-bicyclic framework with a tertiary amide rotational angle (ω) of >140° (i.e., akin to structure **1a** rather than **1b**). In general, the rotational freedom of peptide bonds is restricted due to the partial double bond character of the CO–NH secondary amide, which results in a high-energy barrier for *cis* → *trans* isomerisation. However numerous studies have shown that *N*-acylated prolines (general structure **5a**) are unique amongst aminoacids in that they exist as readily interconvertable mixtures of *cis* and *trans* isomers about the CO–N tertiary amide bond.[8] We considered the possibility that the rotational freedom present in *N*-acylprolines may be mirrored in scaffolds **1** and **2** which also contain the tertiary amide bond. Therefore, a range of design strategies were examined with the potential to restrict rotational freedom about the CO–N bond within **1** and **2** and confine the

Keywords: Cysteinyl proteinase inhibitor; Heterobicyclic ketone.
* Corresponding author. Tel.: +44 1223 264211; fax: +44 1223 265662; e-mail: martin.quibell@incenta.co.uk
† Current address: Siena Biotech S. p.A., Via Fiorentina, 1 53100 Siena, Italy.

0960-894X/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2005.01.022

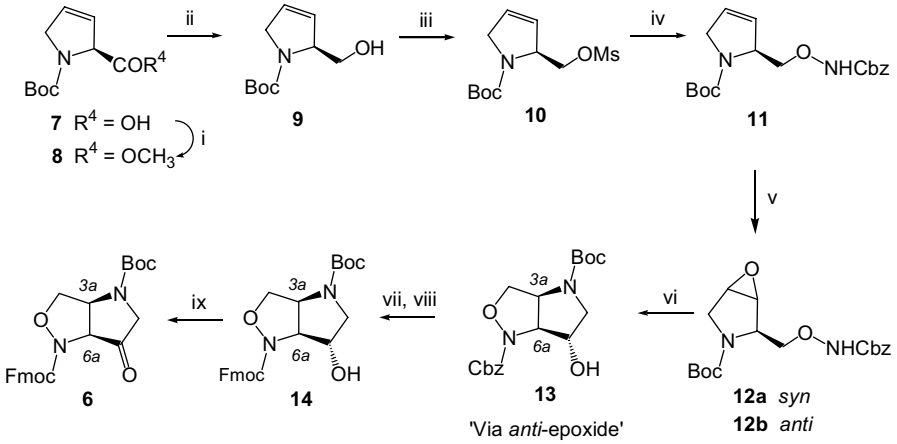


1a (3a*S*, 6a*R*) ω = 180° **1b** (3a*S*, 6a*R*) ω = 0° **2** (3a*R*, 6a*S*) **3** (3a*S*, 6a*S*) X = O **4** (3a*R*, 6a*S*) X = NH **5a** Y = CH_2 **b** Y = O **c** Y = NH

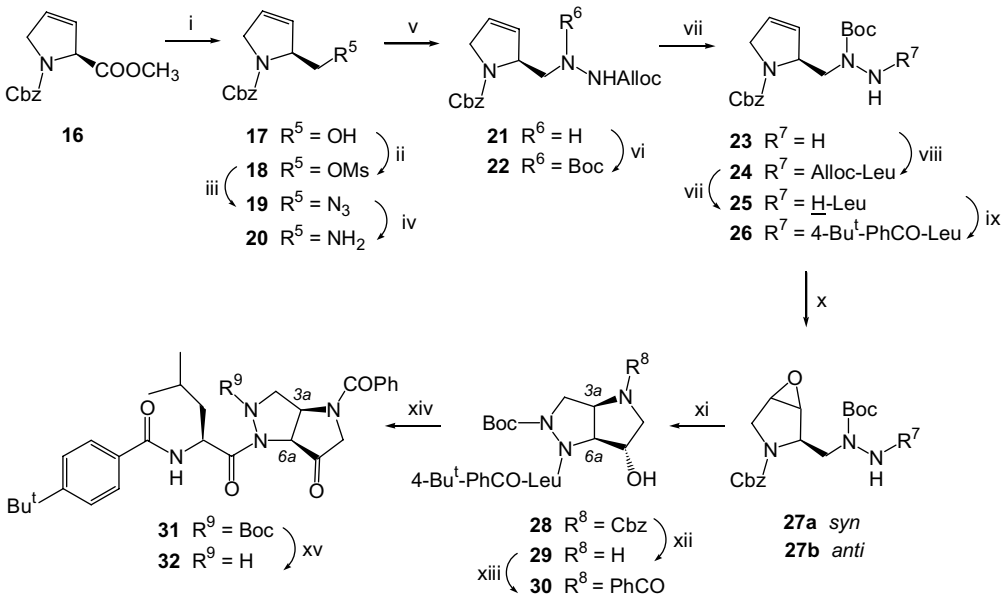
ω-bond angle close to the predicted bioactive conformation.[9] Initially we considered introduction of steric bulk to the δ-position of the bicyclic scaffold (see scaffolds **1** and **2**) to spatially occlude rotation about the CO–N bond. However, this was dismissed because we had previously shown that the δ-methylene protons and the α-proton of the P2 leucine residue are only 2.5 Å apart in the predicted bioactive conformation.[6] Thus, even simple δ-methylation could have a deleterious effect by inducing a rotation in the psi (ψ) angle of the P2 leucine within the inhibitor into a sub-optimal binding conformation. Alternatively, we were intrigued by the literature precedence for heteroatom substitution of the δ-methylene as a method for restricting rotational freedom in *N*-acylated prolines.[10,11] Literature clearly demonstrates that *N*-acetyl-5-oxo-proline (**5b**, $R^1 = CH_3$, $R^3 = OH$) exists almost exclusively *trans* ($\omega \sim 180°$) at neutral pH whereas *N*-acetyl-proline (**5a**, $R^1 = CH_3$, $R^3 = OH$) forms a 1:1 *cis*/*trans* mixture.[11] A simplistic explanation for the predominant *trans* conformation of **5b** is that the carbonyl oxygen and 5-oxo-oxygen would be eclipsed in the *cis*-conformation, which through electron/electron repulsion provides a high energy barrier to rotation. Theoretically, a similar conclusion may be drawn for *N*-acetyl-5-aza-proline (**5c**, $R^1 = CH_3$, $R^3 = OH$), although experimentally this has not been shown to date. We were hopeful that the observed stabilisation of the *trans*-amide in *N*-acetyl-5-hetero-prolines may provide a corresponding effect when incorporated into the new *cis*-6-oxo-hexahydro-2-oxa-1,4-diazapentalene **3** and *cis*-6-oxo-hexahydropyrrolo[3,2-*c*]pyrazole **4** scaffolds. In this context, we have now examined the effects on inhibitor potency against CAC1 proteinases for compounds **15** and **32** derived from scaffolds **3** and **4**, respectively.

Synthesis of the *cis*-hexahydropyrrolo[3,2-*b*]pyrrol-3-one scaffold **2** has previously been described in detail and utilises an intramolecular cyclisation of a tethered amine epoxide to control stereochemistry during formation of the bicyclic framework.[5] We envisaged that an analogous strategy would provide compounds derived from bicyclic scaffolds **3** and **4** (Schemes 1 and 2).[12]

Synthesis of protected ketone **6**, a building block that was suitable for solid phase syntheses of analogues of scaffold **3**,[5,12] commenced from the available Boc–dehydroproline **7**. Conversion of acid **7** to methyl ester **8** (88%) was followed by reduction to alcohol **9** (92%). Conversion of hydroxyl **9** to mesylate **10** (100%), provided a crude oil that was used without further purification. Deprotonation of benzyl *N*-hydroxycarbamate (Cbz-NHOH) provided the anion for nucleophilic displacement of mesylate **10**, which after heating at 65 °C overnight and following silica gel purification gave the desired aminooxymethyl intermediate **11** (26%)[13] and recovered mesylate (47%). Epoxidation of alkene **11** provided **12** as a mixture of *syn*-**12a** and *anti*-**12b** epoxides (62%) that co-eluted upon silica chromatography and exhibited complex proton NMR spectra. We envisaged that within epoxide mixture **12** only *anti*-**12b** could cyclise because potential cyclisation of the *syn*-epoxide **12a** would lead to the thermodynamically disfavoured *trans*-5,5-bicycle.[5] Indeed, treatment of mixture **12** with potassium carbonate in acetonitrile, followed by purification over silica gel, led to the recovery of *syn*-epoxide



Scheme 1. Synthesis of (3a*S*,6a*S*)-**6**. Reagents and conditions: (i) ethereal CH_2N_2, −15 °C—rt; (ii) $LiBH_4$, MeOH, THF; (iii) methanesulfonyl chloride, pyridine, DCM; (iv) Cbz-NH-OH, NaH, THF, 65 °C; (v) *m*-chloroperoxybenzoic acid, DCM; (vi) potassium carbonate, CH_3CN; (vii) Pd–C, H_2, ethanol; (viii) 1.05 equiv Fmoc–Cl, 2.1 equiv Na_2CO_3, 1,4-dioxane, water; (ix) Dess–Martin periodinane, DCM.

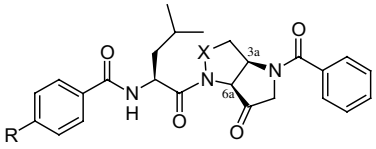


Scheme 2. Synthesis of (3a*R*,6a*S*)-*N*-[(1*S*)-1-(4-benzoyl-6-oxo-hexahydro-pyrrolo[3,2-*c*]pyrazole-1-carbonyl)-3-methyl-butyl]-4-*tert*-butylbenzamide **32**. Reagents and conditions: (i) $LiBH_4$, MeOH, THF; (ii) methanesulfonyl chloride, triethylamine, DCM; (iii) sodium azide, DMF, 110 °C; (iv) Ph_3P/H_2O, 1,4-dioxane, 50 °C; (v) 3-phenyloxaziridine-2-carboxylic acid allyl ester, DCM; (vi) $(Boc)_2O$, triethylamine/MeOH, 60 °C; (vii) $Pd(PPh_3)_4$, $PhSiH_3$, DCM; (viii) Alloc-Leu-F, DMF; (ix) 4-*tert*-butylbenzoic acid, HBTU, HOBT, NMM, DMF; (x) *m*-chloroperoxybenzoic acid, DCM; (xi) potassium carbonate, CH_3CN, 60 °C; (xii) Pd–C, H_2, ethanol; (xiii) $(PhCO)_2O$, DMF; (xiv) Dess–Martin periodinane, DCM; (xv) TFA.

12a (25%), together with a new compound that was less mobile on TLC and identified as the *cis*-heterobicycle **13** (50%). Removal of Cbz protection was followed by re-protection with 9-fluorenylmethoxycarbonyl chloroformate (Fmoc–Cl), providing bicyclic alcohol **14** (75%). As the final step in Scheme 1, alcohol **14** was smoothly oxidised by Dess–Martin periodinane to provide target bicyclic ketone **6** (80%) as a white solid.[14] With ketone **6** in hand, a solid phase synthesis, analogous to that previously described for bicycles **1** and **2**,[5,6,12] was undertaken to give (3a*S*,6a*S*)-*N*-[(1*S*)-1-(4-benzoyl-6-oxo-hexahydro-2-oxa-1,4-diaza-pentalene-1-carbonyl)-3-methyl-butyl]-4-dimethylamino benzamide **15** (Table 1).[15]

Next we undertook the synthesis of inhibitor **32**, an analogue of scaffold **4**, entirely in solution (Scheme 2). Synthesis commenced from Cbz-dehydroproline methyl ester **16**, which was reduced to alcohol **17** (90%). Conversion to mesylate **18** (95%) provided a crude oil that was used without further purification. Surprisingly, all attempts to directly prepare protected hydrazide **21** through displacement of mesylate **18** with allyloxycarbonylhydrazide[16] unexpectedly failed. Numerous solvents and reaction conditions were examined, but in each case either no reaction or an intractable mixture was obtained. Therefore, we explored other strategies towards hydrazide **21** and successfully adapted the electrophilic *N*-amination methods of Niederer et al., used to prepare protected hydrazino acids from the corresponding aminoacids.[17] Thus, treatment of mesylate **18** with sodium azide in DMF gave azidomethyl intermediate **19** (72%), which was reduced to amine **20** (74%) following the general methods of Mandville et al.[18] Treatment of amine **20** with *N*-Alloc-3-phenyloxaziridine[17] in DCM afforded the desired hydrazide **21** as a pale yellow oil (36%), which was *N*-Boc substituted to give the tri-orthogonal protected **22** (79%). Unfortunately,

Table 1. Preliminary inhibitory activities (K_i^{ss}, nM) for 5,5-bicyclic inhibitors **15** and **32** and the equivalent δ-methylene bicycles derived from scaffold **2**[5,21] against CAC1 proteinases (mean of $n = 3$ determinations). Assay conditions are as detailed previously[12]



Compound	Cat. K	Cat. L	Cat. S	Cat. B	Cruz.	CPB
15 (X = O, R = Me_2N)	40.1 ± 16.7	>4300	>3000	>6000	2500 ± 1500	1000 ± 600
32 (X = NH, R = Bu^t)	3.5 ± 0.1	370 ± 80	434 ± 142	119 ± 12	93.2 ± 31.7	49.5 ± 23.6
33[21] (X = CH_2, R = Me_2N)	5.5 ± 2.0	1800 ± 630	>3000	>10,000	600 ± 350	1170 ± 540
34[5] (X = CH_2, R = Bu^t)	10.1 ± 6.7	>3500	>4500	>10,000	173 ± 86	691 ± 350

the analogous epoxidation and base catalysed ring-closure described earlier (**11** → **13**) was not an option for intermediate **22** due to the complicating presence of the Alloc protecting group. Thus, removal of Alloc protection following the general conditions described by Dessolin et al.[19] provided hydrazide **23** (97%) that was treated with the acyl fluoride of Alloc-leucine[5] to obtain acylated intermediate **24** (63%). Removal of Alloc protection[19] (96%) was followed by acylation of amine **25** with 4-*tert*-butylbenzoic acid using standard uronium activation chemistries to give substituted hydrazide **26** (84%). Epoxidation of **26** now proceeded smoothly to give a mixture of *syn*-**27a** and *anti*-**27b** (62%). In an analogous manner to that described earlier for epoxide mixture **12**, we envisaged that only *anti*-**27b** would cyclise. Thus, treatment of epoxide mixture **27** with potassium carbonate in acetonitrile, followed by purification over silica gel, gave a new compound that was less mobile on TLC and was identified as the *cis*-heterobicycle **28** (42%). The Cbz group was then replaced with a benzoyl group to obtain the Boc-protected alcohol **30** (two steps 68%). Subsequent oxidation of **30** with Dess–Martin periodinane provided *N*-Boc protected bicyclic ketone **31** (81%),[20a] which was treated with trifluoroacetic acid to give the final fully deprotected inhibitor **32** (37%).[20b]

Bicyclic inhibitors **15** and **32** were screened against cathepsins K, L, S and B as well as the parasitic proteinases cruzain and CPB.[12] Preliminary steady-state inhibition constants (K_i^{ss}) are shown in Table 1 (mean of $n = 3$ determinations). The substituents detailed in Table 1 were chosen to provide a direct comparison with our previously detailed bicyclic inhibitors **33**[21] and **34**.[5]

Table 1 shows that the new heterobicyclic inhibitors derived from scaffolds **3** and **4** provide low nanomolar inhibitors of human cathepsin K and have the potential, when substituted with appropriate binding elements, to inhibit other CAC1 proteinases.[5] For the cathepsin K inhibitors disclosed, the δ-methylene (**33**) to δ-oxygen (**15**) modification has given an approximately 8-fold loss in potency. However, the δ-methylene (**34**) to δ-nitrogen (**32**) modification has given an across the board increase in potency ranging from approximately 3-fold for cathepsin K to 14-fold for *Leishmania mexicana* CPB and greater than 80-fold for cathepsin B. The inhibition kinetics for inhibitor **32** are fully reversible against each proteinase. One possible inference for this universal increase in potency is that the design process detailed herein for restricting rotational (and therefore conformational) freedom about the CO–N tertiary amide bond has, in the case of inhibitor **32** been successful. However, inferences of binding modes based solely upon changes in potency are an over simplification. The introduction of the δ-heteroatom into scaffold **2** may induce numerous effects such as changes in ring geometry and puckering of the bicyclic framework, any of which may influence presentation of the electrophilic ketone to the active site thiol and alter potency.

In summary, a restricted rotation design process applied to *cis*-5,5-bicyclic scaffold **2** indicated that new heterobicycles **3** and **4** may provide improved potency against CAC1 proteinases. Synthetic routes were successfully devised and example heterobicyclic inhibitors **15** and **32** prepared. Compound **32** was designed with appropriate binding moieties for cathepsin K inhibition,[5] but shows a significant increase in potency against all CAC1 proteinases examined. This suggests that the heterobicyclic framework defined in scaffold **4** may indeed be rotationally (conformationally) restricted when compared to the equivalent methylene scaffold **2**, but still able to access the bioactive conformational space. Therefore scaffold **4** has the potential to give high potency inhibitors of CAC1 proteinases.

Acknowledgements

The authors wish to thank Cambridge University Chemistry Department for compound analyses and Mr. Mark Sleeman of University of Oxford for optical rotation data.

References and notes

1. Barrett, A. J.; Rawlings, N. D.; Woessner, J. F. *Handbook of Proteolytic Enzymes*; Academic: New York, 1998.
2. Lecaille, F.; Kaleta, J.; Brömme, D. *Chem. Rev.* **2002**, *102*, 4459.
3. Brömme, D.; Kaleta, J. *Curr. Pharm. Des.* **2002**, *8*, 1639.
4. Sajid, M.; McKerrow, J. H. *Mol. Biochem. Parasitol.* **2002**, *120*, 1.
5. For example, see Quibell, M.; Benn, A.; Flinn, N.; Monk, T.; Ramjee, M.; Ray, P.; Wang, Y.; Watts, J. *Bioorg. Med. Chem.* **2005**, *13*, 609, and references cited therein.
6. Quibell, M.; Benn, A.; Flinn, N.; Monk, T.; Ramjee, M.; Wang, Y.; Watts, J. *Bioorg. Med. Chem.* **2004**, *12*(21), 5689.
7. Fenwick, A. E.; Gribble, A. D.; Ife, R. J.; Stevens, N.; Witherington, J. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 199.
8. For example, see: Fischer, S.; Dunbrack, R. L., Jr.; Karplus, M. *J. Am. Chem. Soc.* **1994**, *116*, 11931.
9. In-house molecular modelling was performed as previously described in detail using WebLab ViewerPro (http://www.accelrys.com): see, Watts, J.; Benn, A.; Flinn, N.; Monk, T.; Ramjee, M.; Ray, P.; Wang, Y.; Quibell, M. *Bioorg. Med. Chem.* **2004**, *12*, 2903.
10. Shireman, B. T.; Miller, M. J.; Jonas, M.; Wiest, O. *J. Org. Chem.* **2001**, *66*, 6046.
11. Galardy, R. E.; Alger, J. R.; Liakopoulou-Kyriakides, M. *Int. J. Pept. Protein Res.* **1982**, *19*, 123.
12. Full experimental protocols for Schemes 1 and 2 are described in: Quibell, M.; Ray, P. C.; Watts, J. P. WO Patent 04007501. Within this patent see syntheses for **6** (pp 416–423); **15** (p 415); **22** (pp 426–432); **32** (pp 433–437) and enzymatic methods (pp 531–543).
13. Data for intermediate **11**: TLC (single spot, $R_f = 0.35$, EtOAc–hexane 2:3). Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.05; H, 6.94; N, 8.04, found C, 62.18; H, 7.05; N, 7.90. Exact mass calcd for $C_{18}H_{24}N_2O_5$ (MNa^+): 371.1583, found 371.1590 (δ +1.83 ppm); $[\alpha]_D^{22}$ −42.4 (*c* 0.663, $CHCl_3$).
14. Data for ketone **6**: TLC (single spot, $R_f = 0.30$, EtOAc–heptane 2:3). Exact mass calcd for $C_{25}H_{26}N_2O_6$ (MNa^+): 473.1689, found 473.1690 (+0.24 ppm); $[\alpha]_D^{22}$ −92.4 (*c* 0.224, $CHCl_3$). δ_H (300 K, 500 MHz, $CDCl_3$) mixture of rotamers major:minor 1.5:1, 1.48 (s, $C(CH_3)_3$, 5.4H), 1.50 (s, $C(CH_3)_3$, 3.6H), 3.49–3.58 (m, BocNCHCH_2, 1H),

3.78–3.92 (m, BocNCH_2, 2H), 4.13 (d, J = 9.5 Hz, BocNCHCH_2, 0.4H), 4.20–4.29 (m, Fmoc–CH and BocNCHCH_2, 1.6H), 4.46–4.52 (m, Fmoc–CH_2, 1H), 4.60–4.74 (m, Fmoc–CH_2, FmocNCH, BocNCH, 2.4H), 4.83 (dd, J = 7.5 and 4.3 Hz, BocNCH, 0.6H), 7.29–7.78 (aromatic, 8H); δ_C (300 K, 125 MHz, $CDCl_3$) 28.38, 28.31 (C(CH_3)$_3$), 46.96, 47.05 (Fmoc–CH), 52.40, 52.93 (BocNCH$_2$), 61.95 (BocNCH), 64.48, 65.31 (FmocNCH), 68.59, 68.76 (Fmoc–CH$_2$), 77.17, 77.31 (BocNCHCH$_2$), 81.61 (C(CH$_3$)$_3$), 120.02, 125.11, 125.35, 127.21, 127.28, 127.98 (Fmoc aromatic CH), 141.29, 141.33, 143.04, 143.12 (Fmoc quaternary), 153.09, 154.00, 157.64 (Boc and Fmoc C=O), 204.85, 205.44 (C=O).

15. Data for inhibitor **15**: analytical RP-HPLC t_R = 13.18 min (major peak > 95% by UV 215 nm, Phenomenex Jupiter C_4, 5 μm, 300 Å, 250 × 4.6 mm, acetonitrile–water gradient). Exact mass calcd for $C_{27}H_{32}N_4O_5$ (MNa^+): 515.2265, found 515.2285 (+3.96 ppm).
16. Prepared following, Macleay, R. E.; Lange, H. C. U.S. Patent 5,338,853, 1994.
17. Niederer, D. A.; Kapron, J. T.; Vederas, J. C. *Tetrahedron Lett.* **1993**, *34*(43), 6859.
18. Mandville, G.; Ahmar, M.; Bloch, R. *J. Org. Chem.* **1996**, *61*, 1122.
19. Dessolin, M.; Guillerez, M.-G.; Thieriet, N.; Guibe, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*(32), 5741.
20. (a) Data for ketone **31**: TLC (single spot, R_f = 0.65, EtOAc–heptane 3:2). Exact mass calcd for $C_{34}H_{44}N_4O_6$ (MNa^+): 627.3153, found 627.3167 (+2.26 ppm); (b) Data for inhibitor **32**: TLC (single spot, R_f = 0.26, EtOAc–heptane 3:1); analytical RP-HPLC t_R = 18.10–19.70 min (broad major peak > 95% by UV 215 nm, Phenomenex Jupiter C_4, 5 μm, 300 Å, 250 × 4.6 mm, acetonitrile–water gradient). Exact mass calcd for $C_{29}H_{36}N_4O_4$ (MNa^+): 527.2629, found 527.2619 (−1.78 ppm); $[\alpha]_D^{22}$ −168.2 (c 0.022, $CHCl_3$).
21. Prepared as detailed in Ref. 5. Data for inhibitor **33**: TLC (single spot, R_f = 0.25, EtOAc–heptane 9:1). Exact mass calcd for $C_{28}H_{34}N_4O_4$ (MNa^+): 513.2478, found 513.2492 (+2.72 ppm); $[\alpha]_D^{22}$ −38.4 (c 0.503, $CHCl_3$).