Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 2585–2588

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Asymmetric Synthesis of BB-3497—A Potent Peptide Deformylase Inhibitor

Lisa M. Pratt,* R. Paul Beckett, Stephen J. Davies, Steven B. Launchbury, Andrew Miller,† Zoe M. Spavold, Richard S. Todd and Mark Whittaker

British Biotech Pharmaceuticals Limited, Watlington Road, Cowley, Oxford OX4 6LY, UK

Received 18 April 2001; revised 26 June 2001; accepted 18 July 2001

Abstract—By screening a library of metalloenzyme inhibitors, the *N*-formyl-hydroxylamine derivative BB-3497 was identified as a potent inhibitor of *Escherichia coli* peptide deformylase with antibacterial activity both in vitro and in vivo. The homochiral synthesis of BB-3497, involving a novel asymmetric Michael addition reaction is described.

Introduction

All ribosome-mediated synthesis of proteins starts with a methionine residue. In bacteria and the organelles of eukaryotes, the amino group of the methionyl moiety carried by the initiator $tRNA^{fMet}$ is *N*-formylated by formyltransferase prior to its incorporation into a polypeptide.[1] This is thought to be required for proper protein synthesis initiation. Consequently, *N*-formylmethionine is always present at the N-terminus of a nascent bacterial polypeptide. However, in bacteria most mature proteins do not retain the *N*-formyl group or the terminal methionine residue. Following translation, the formyl group is hydrolysed by the metalloenzyme peptide deformylase (PDF, EC 3.5.1.31), which is necessary before further processing at the N-terminus by methionine aminopeptidase (EC 3.4.11.18) can take place. Deformylation is therefore a crucial step in bacterial protein biosynthesis and PDF is essential for bacterial growth. The gene encoding PDF (*def*) is present in all sequenced pathogenic bacterial genomes, making it an attractive target for antibacterial chemotherapy.[2] It has been generally accepted that eukaryotic organelles do not have a deformylase activity. However, very recently a human PDF homologue was identified which is presumed to be involved in deformylation in the mitochondrion.[3] The function and sub-cellular location of this putative gene product have yet to be confirmed. There have been several reports describing inhibitors of *Escherichia coli* peptide deformylase,[4–11] but few of these possess significant antibacterial activity. The naturally occurring antibiotic actinonin, **1**, for which the molecular target was previously unknown, has also been shown to be a potent inhibitor of PDF.[12]

By screening a proprietary library of potential metalloenzyme inhibitors we identified the *N*-formyl-hydroxylamine derivative BB-3497, **2**, as a potent and selective PDF inhibitor with moderate antibacterial activity and in vivo efficacy.[13] The structural relationship between actinonin, BB-3497 and a typical PDF substrate is illustrated (Fig. 1). BB-3497 was originally prepared in a non-stereoselective manner and the absolute stereochemistry was tentatively assigned on the basis of matrix metalloproteinase (MMP) inhibitory activity. Here we describe the asymmetric synthesis of BB-3497 and proof of stereochemistry by small molecule X-ray crystallography.

Chemistry and Enzyme Inhibitory Activity

BB-3497 was initially synthesised by the method of Roques et al. (Scheme 1),[14] whereby the β-(*N*-hydroxy)-amino acid fragment **5** was prepared by conjugate addition of *O*-benzyl-hydroxylamine to 2-*n*-butyl acrylic acid, **4**. Peptide coupling to L-*tert*-leucine *N*,*N*-dimethylamide, followed by formylation afforded a mixture of protected *N*-formyl-hydroxylamine derivatives **RS-6** and **SS-6,** which were separated by column chromatography

*Corresponding author. Fax: +44-1865-780814; e-mail: ayscough@britbio.co.uk

†Present address: Vertex Pharmaceuticals (Europe) Limited, 88 Milton Park, Abingdon, Oxford OX14 4RY, UK.

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00509-1

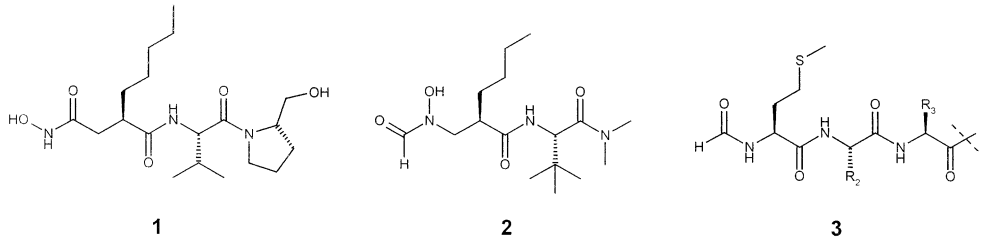
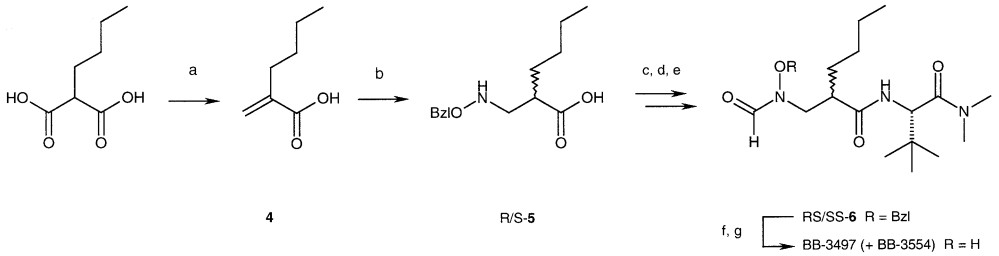


Figure 1. Structural relationship between actinonin (**1**), BB-3497 (**2**) and a generic PDF substrate (**3**).



Scheme 1. Non-asymmetric synthesis of BB-3497 (see ref 14). Reagents and conditions: (a) piperidine, HCHO, EtOH, 80 °C o/n; (b) H_2NOBzl, 80 °C, o/n; (c) HCOOH, Ac_2O; (d) pentafluorophenol, EDC, CH_2Cl_2; (e) H-TleNMe$_2$, DMF, 35 °C; (f) separate diastereoisomers (flash chromatography); (g) H_2, Pd/C, EtOH.

and individually deprotected by hydrogenolysis to provide BB-3497 (from less polar diastereoisomer of **6**) and BB-3554 (from more polar diastereoisomer of **6**). The absolute stereochemistry of these compounds was unknown, but was inferred by studying their matrix metalloproteinase inhibitory activity (Table 1). It was clear that although BB-3497 had relatively weak activity against the MMP enzyme family it was more potent than BB-3554. Since it is well known that the preferred P1′ stereochemistry for MMP inhibition is *R*, corresponding to a natural L-amino acid in the substrate,[15] we tentatively assigned BB-3497 as *N*-[2*R*-(*N*-formyl-*N*-hydroxy-aminomethyl)-hexanoyl]-*S*-*tert*-leucine *N*,*N*-dimethylamide. It is also noteworthy that while BB-3497 is equipotent with actinonin against *E. coli* PDF, it is significantly less potent against several of the MMP enzymes, and hence more selective. This observation appears to be in general for the *N*-formyl-hydroxylamine class and may offer some advantages in a clinical setting.

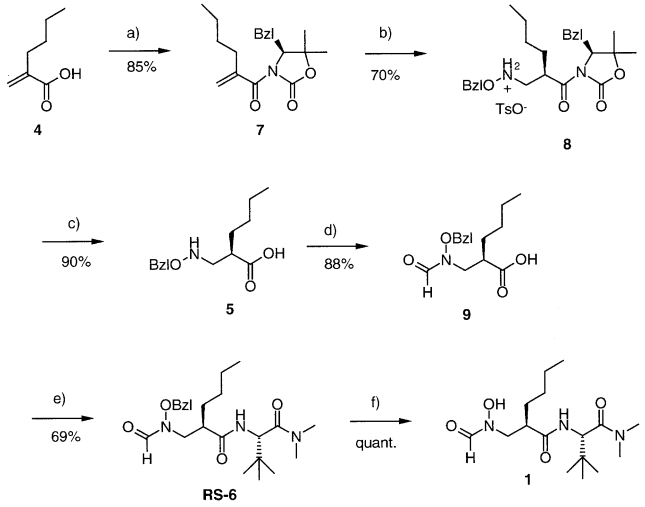
In order to provide sufficient BB-3497 for detailed biological evaluation, and to facilitate an analogue synthesis programme, we sought an asymmetric synthetic route to the β-(*N*-hydroxy)amino acid derivative **5**. At the time we were unaware of any approaches to this class of compound in the literature.[16] Amongst a number of possible approaches that we investigated was the asymmetric counterpart of the conjugate addition described above, in which the new asymmetric centre was generated by a highly stereoselective protonation step (Scheme 2).

The homochiral acrylate **7** was prepared under standard conditions via the mixed anhydride. We found that addition of *O*-benzyl-hydroxylamine to **7** *at ambient temperature* proceeded in >90% d.e. and that crystallisation as the tosyl salt afforded a single diastereoisomer of the Michael adduct, **8**.[17] The (*S*)-benzyl Superquat™ chiral auxiliary[18] was preferred over the conventional (4*S*)-benzyloxazolidin-2-one chiral auxiliary of Evans[19] because of its lower propensity to undergo oxazolidinone ring opening. After liberation of the free base, removal of the Superquat™ auxiliary was performed under the less stringent conditions described by Davies et al.[18] We chose to formylate **5** before coupling to the *tert*-leucine fragment because the intermediate **9** could be used for synthesis of a wide variety of P2′ and P3′ modified analogues as well as BB-3497 itself. The original assignment of stereochemistry of BB-3497 was supported by a small molecule crystal structure of the benzyl precursor **RS-6**,[20] (Fig. 2) and later by the crystal structure of *E. coli* PDF/BB-3497 complex.[13] The stereochemical outcome of the conjugate addition

Table 1. Metalloenzyme inhibition profile of BB-3497, BB-3554 and actinonin against *E. coli* PDF.Ni, matrix metalloproteinases (MMP), neutral endopeptidase (NEP; enkephalinase) and angiotensin converting enzyme (ACE)

	IC_{50} (nM)[a]						
	PDF.Ni	MMP-1	MMP-2	MMP-3	MMP-7	NEP	ACE
BB-3497	7	2000	15000	>100,000	>100,000	50,000	>100,000
BB-3554	70	>100,000	>100,000	>100,000	>100,000	ND[c]	ND[c]
Actinonin	10	1100	3000	6000	60% I[b]	6700	>100,000

[a]The IC_{50} represents the concentration of inhibitor (nM) required to decrease enzyme activity by 50%.
[b]% inhibition at 100 μM.
[c]Not determined.



Scheme 2. Asymmetric synthetic route to BB-3497. Reagents and conditions: (a) *t*BuCOCl, Et_3N then 3-lithio-4-benzyl-5,5-dimethyl-oxazolidin-2-one, THF, −78 °C; (b) (i) H_2NOBzl, rt, o/n; (ii) *p*TsOH, EtOAc; (c) (i) 1 M Na_2CO_3, EtOAc; (ii) LiOH, aq THF, 0 °C; (d) HCOOAc, THF; (e) H-TleNMe$_2$, HOAt, EDC, DMF; (f) H_2, Pd/C, EtOH.

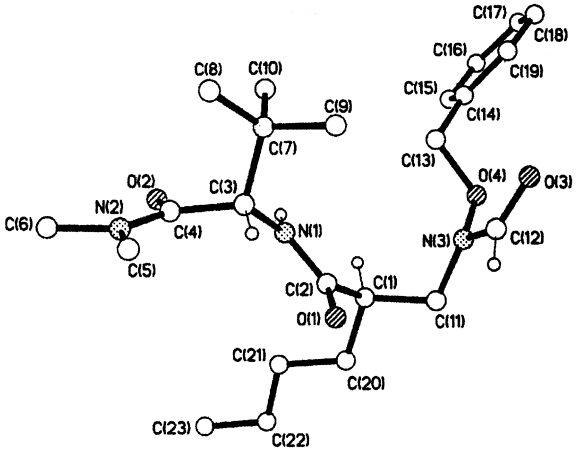


Figure 2. X-ray crystal structure of **RS-6**.

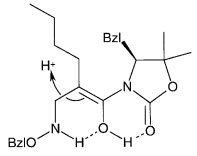


Figure 3. Chiral enolate in conjugate addition.

reaction is consistent with protonation from the less hindered face of the chiral enolate complex (Fig. 3).

Conclusion

We have identified a novel asymmetric conjugate addition reaction that provides a key intermediate required in the synthesis of homochiral BB-3497. The full scope of this reaction has yet to be explored. The absolute stereochemistry of BB-3497 corresponds to that of a natural formyl-methionyl peptide substrate of PDF. Modification of the *tert*-leucine amide fragment of BB-3497 to provide compounds with substantially improved antibacterial activity will be reported elsewhere.

Acknowledgements

We wish to thank Professor Bill Clegg and Dr Mark Elsegood, University of Newcastle, for the small molecule crystal structure determination and Wayne Thomas, British Biotech Pharmaceuticals Limited, for the biological screening.

References and Notes

1. Adams, J. M.; Capecchi, M. *Proc. Natl. Acad. Sci. U.S.A.* **1966**, *55*, 147.
2. Giglione, C.; Pierre, M.; Meinnel, T. *Mol. Microbiol.* **2000**, *36*, 1197.
3. Giglione, C.; Serero, A.; Pierre, M.; Boisson, B.; Meinnel, T. *EMBO J.* **2000**, *19*, 5916.
4. Hu, Y. J.; Rajagopalan, P. T.; Pei, D. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2479.
5. Meinnel, T.; Patiny, L.; Ragusa, S.; Blanquet, S. *Biochemistry* **1999**, *38*, 4287.
6. Durand, D. J.; Gordon, G. B.; O'Connell, J. F.; Grant, S. K. *Arch. Biochem. Biophys.* **1999**, *367*, 297.
7. Green, B. G.; Toney, J. H.; Kozarich, J. W.; Grant, S. K. *Arch. Biochem. Biophys.* **2000**, *375*, 355.
8. Huntington, K. M.; Yi, T.; Wei, Y.; Pei, D. *Biochemistry* **2000**, *39*, 4543.
9. Apfel, C.; Banner, D. W.; Bur, D.; Dietz, M.; Hirata, T.; Hubschwerlen, C.; Locher, H.; Page, M. G.; Pirson, W.; Rosse, G.; Specklin, J. L. *J. Med. Chem.* **2000**, *43*, 2324.
10. Jayasekera, M. M.; Kendall, A.; Shammas, R.; Dermyer, M.; Tomala, M.; Shapiro, M. A.; Holler, T. P. *Arch. Biochem. Biophys.* **2000**, *381*, 313.
11. Wei, W.; Tian, Y.; Huntingdon, K. M.; Chaudhury, C.; Pei, D. *J. Combinatorial Chem.* **2000**, *2*, 650.
12. Chen, D. Z.; Patel, D. V.; Hackbarth, C. J.; Wang, W.; Dreyer, G.; Young, D. C.; Margolis, P. S.; Wu, C.; Ni, Z. J.; Trias, J.; White, R. J.; Yuan, Z. *Biochemistry* **2000**, *39*, 1256.
13. Clements, J. M.; Beckett, P.; Brown, A.; Catlin, G.; Lobell, M.; Palan, S.; Thomas, W.; Whittaker, M.; Baker, P. J.; Rodgers, F.; Barynin, V.; Rice, D. W.; Hunter, M. G. *Antimicrob. Agents Chemother.* **2001**, *45*, 563.

14. Fournie-Zaluski, M.-C.; Coulaud, A.; Bouboutou, R.; Chaillet, P.; Devin, J.; Waksman, G.; Costentin, J.; Roques, B. P. *J. Med. Chem.* **1985**, *28*, 1158.
15. Johnson, W. H.; Roberts, N. A.; Borkakoti, N. *J. Enzyme Inhib.* **1987**, *2*, 1.
16. Recently an optical resolution method has been described for a related compound: Robl, J. A.; Simpkins, L. M.; Asaad, M. M. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 257.
17. A mixture of acrylate **7** (63 mmol) and *O*-benzylhydroxylamine (126 mmol) was stirred at ambient temperature o/n. The crude mixture was dissolved in ethyl acetate and washed sequentially with 1 M HCl, 1 M Na_2CO_3 and brine. The organics were dried over anhydrous $MgSO_4$ and the solvent removed in vacuo to obtain a yellow oil (59 mmol). This was treated with 1 equiv *p*-toluene sulfonic acid and crystallised from ethyl acetate to obtain the Michael adduct **8** as a white crystalline solid.
18. Davies, S. G.; Sanganee, H. J. *Tetrahedron: Asymmetry* **1995**, *6*, 671.
19. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737.
20. Crystallographic data for **RS-6** is deposited with the Cambridge Crystallographic Data Centre (Supplementary Publication No: CCDC 160714) and can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; e-mail: deposit@ccdc.cam.ac.uk