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Structure–Activity Relationships of the Peptide Deformylase Inhibitor BB-3497: Modification of the Methylene Spacer and the P1′ Side Chain

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Abstract—Structural modifications to the peptide deformylase inhibitor BB-3497 are described. In this paper, we describe the initial SAR around this lead for modifications to the methylene spacer and the P1′ side chain. Enzyme inhibition and antibacterial activity data revealed that the optimum distance between the *N*-formyl hydroxylamine metal binding group and the P1′ side chain is one unsubstituted methylene unit. Additionally, lipophilic P1′ side chains that closely mimic the methionine residue in the substrate provided compounds with the best microbiological profile.

Ribosomal protein synthesis in bacteria is initiated by *N*-formylation of methionyl-*t*RNA by formyltransferase. Most mature proteins, however, do not retain the *N*-formyl group or the terminal methionine residue. Deformylation is therefore a crucial step in bacterial protein biosynthesis and the metalloenzyme responsible, peptide deformylase (PDF), is essential for bacterial growth.[1] Bacterial PDF is now widely recognised as an attractive target for antibacterial chemotherapy.[2]

We reported previously[3] that the *N*-formyl hydroxylamine **BB-3497** (Fig. 1) is an effective inhibitor ($IC_{50} = 7$ nM) of the *Escherichia coli* PDF·Ni enzyme, exhibiting potent antibacterial activity both in vitro and in vivo. In a subsequent communication,[4] we identified the *N*-formyl hydroxylamine and hydroxamic acid structural motifs to be the optimum metal binding groups on the pseudopeptidic backbone of **BB-3497**. Further to this study, we have conducted a systematic modification of the other sites indicated in Figure 1. In this communication the optimum distance between the metal binding group and the P1′ side chain is evaluated and the preferred P1′ substituents for enzyme binding affinity and antibacterial activity are discussed.

During the course of this investigation, several X-ray crystal structures of PDF enzymes were elucidated.[5–8] The structural information provided by these studies

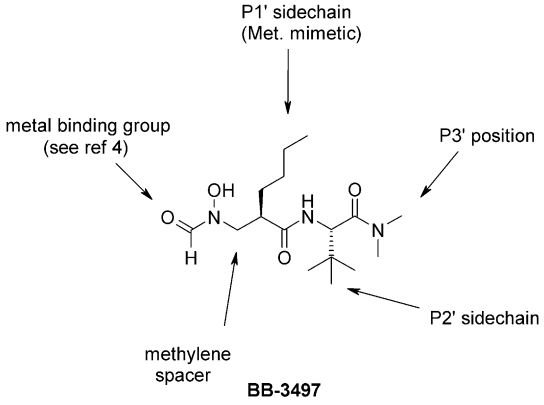

Figure 1. BB-3497 structure–activity relationships.

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was particularly valuable in terms of designing and modelling P1′ side chain modifications of **BB-3497** that could occupy the well-defined S1′ pocket.

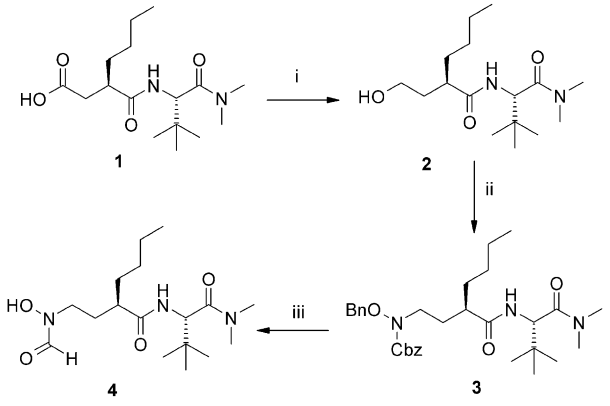
The chemical modifications described here are an attempt to understand the key structural requirements that may lead to molecules with an improved antibacterial profile and pharmacological properties.

Chemistry

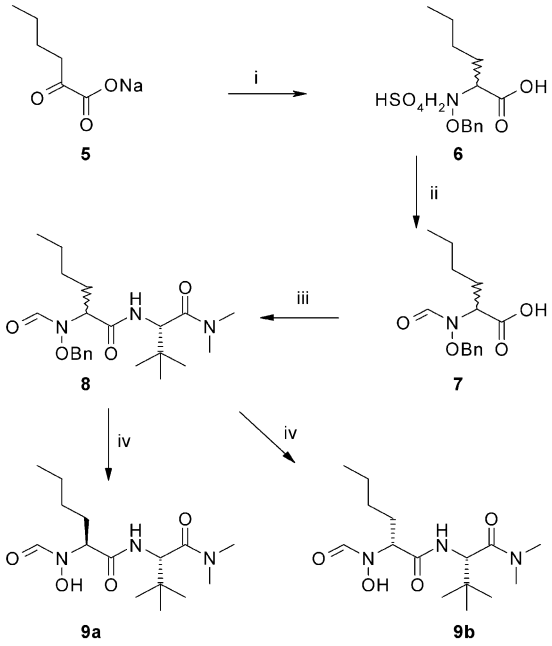
To examine the optimal distance between metal binding group (MBG) and the P1′ position (Table 1), three different approaches were used. The first explored route involved the synthesis of compound **4** containing an ethyl spacer and is shown in Scheme 1. Conversion of the carboxylic acid analogue **1**[9] to the mixed anhydride using ethyl chloroformate, followed by reduction with sodium borohydride gave the primary alcohol **2**, which was subjected to Mitsunobu conditions with CbzNHOBn to give *N*,*O*-bisprotected hydroxylamine **3**. Hydrogenolysis of **3** and formylation with *N*-formyl benzotriazole[10] (BtCHO) gave **4** after purification by preparative HPLC.

To further probe the optimum distance between the MBG and the P1′ position, a second approach was based on preparing those compounds which did not contain the methylene spacer group (Scheme 2). Reductive amination of 2-oxo-hexanoic acid–sodium salt **5** with *O*-benzylhydroxylamine and acidic workup with sulfuric acid afforded **6** in excellent yield. Subsequent *N*-formylation using *N*-formyl imidazole (prepared in situ from carbonyl diimidazole and formic acid) and standard peptide coupling conditions using *tert*-leucine-NMe_2[11] gave **8** as a mixture of diastereoisomers. Separation of the isomers by preparative HPLC and removal of the *O*-benzyl protecting group by Pd-catalysed hydrogenation provided the target compounds **9a** and **9b**.

We also prepared an analogue of **BB-3497** containing a methyl group on the methylene spacer in order to evaluate the effect of substitution in this position on enzyme



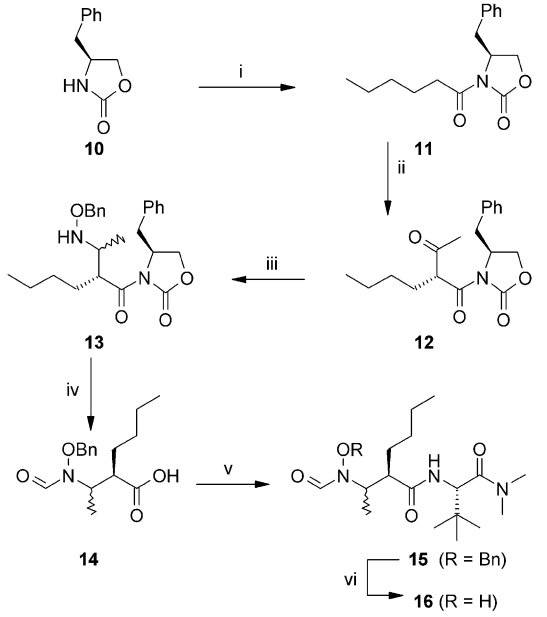
Scheme 1. (i) (a) Ethyl chloroformate, Et_3N; (b) $NaBH_4$, THF, 36%; (two steps); (ii) PPh_3, DIAD, CbzNHOBn, THF, 30%; (iii) (a) H_2, Pd/C; (b) BtCHO, THF, 32% (two steps).



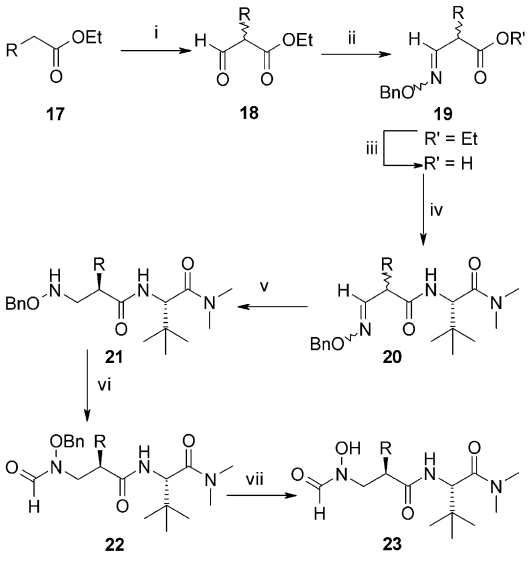
Scheme 2. (i) $BnONH_2$, $NaCNBH_3$, 5 N HCl (pH 4.5), MeOH, rt 82%; (ii) CDI, HCO_2H, Et_2O, rt, 94%; (iii) EDAC·HCl, HOAt, then H-*tert*-leucine NMe_2, DMF, 0 °C to rt, *then* separate diastereoisomers, 45%; (iv) H_2, 10% Pd/C, MeOH, rt, quant.

binding affinity. The non-diastereoselective approach used for the preparation of compound **16** is shown in Scheme 3.

Standard Evans methodology[12] using the (*S*)-oxazolidinone **10** and hexanoyl chloride generated **11** in excellent yield. Acetylation of **11** provided **12** with high diastereoselectivity (>85% d.e.). Reductive amination with



Scheme 3. (i) *n*BuLi, hexanoyl chloride, THF, 86%; (ii) NaHMDS (1 M in THF), acetyl chloride, THF, 80%; (iii) (a) $BnONH_2$·HCl, NaOAc; (b) $NaCNBH_3$, acetic acid, 70% (two steps); (iv) (a) BtCHO, THF, 56%; (b) LiOH, H_2O_2, THF, H_2O, 64%; (v) EDAC·HCl, HOAt, *then* H-*tert*-leucine NMe_2, DMF, 0 °C to rt, 82%; (vi) H_2, 10% Pd/C, MeOH, rt 80%.



Scheme 4. (i) HCOOEt, NaOEt, 23–45%; (ii) NH_2OBn, NaOAc, aq EtOH, 40–70%; (iii) NaOH, MeOH; quant; (iv) H-*tert*-leucine-NMe_2, EDC, HOAt, DMF, 45–75%; (v) $NaCNBH_3$, AcOH, *then* separate diastereoisomers, 30–45%; (vi) BtCHO, THF; 86%; (vii) H_2, Pd/C, MeOH, quant.

O-benzyl hydroxylamine and sodium cyanoborohydride gave **13** as a mixture of diastereoisomers. Subsequent *N*-formylation followed by oxidative hydrolysis of the chiral auxilliary gave the carboxylic acid precursor **14**. Standard coupling conditions using *tert*-leucine-NMe_2[11] provided the compound **15**. Hydrogenolysis under standard conditions afforded the *N*-formyl hydroxylamine **16** as a mixture of diastereoisomers.

To examine the effect of P1′ side-chain modification on PDF enzyme activity and antibacterial activity, a non-stereospecific route was developed to access analogues of **BB-3497** as shown in Scheme 4.

The compounds detailed in Table 2 (**23a–w**) were prepared from the corresponding ethyl esters **17**, with the P1′ side chain already in place. Formylation of **17** with ethyl chloroformate/sodium ethoxide to give the aldehyde **18**, followed by oxime formation using *O*-benzylhydroxylamine in the presence of sodium acetate provided **19**. Saponification of the ethyl ester was followed by amide coupling with *tert*-leucine-NMe_2[11] to give **20** in good yield. Reduction of the oxime using sodium cyanoborohydride provided the protected hydroxylamine as a mixture of diastereoisomers which were separated by chromatography to give the desired (*R*)-diastereoisomer **21**.[13] Hydroxylamine **21** was *N*-formylated[10] using BtCHO and subsequent removal of the *O*-benzyl protecting group by catalytic hydrogenation afforded the target compound **23**. A more detailed procedure for the preparation of the majority of compounds in this paper is available in the patent literature.[14]

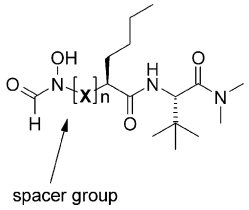
Results and Discussion

All compounds were screened for activity against the PDF.Ni enzyme and selected Gram-positive and Gram-negative bacterial strains.[15]

Any attempt at increasing the length of the spacer group (**4**) or incorporating even a small substituent (**16**) resulted in loss of activity, relative to **BB-3497** (Table 1). Further modification, by complete removal of the spacer group (**9a + 9b**) showed significant loss of PDF enzyme activity. The rigid geometry around the metal binding group and the distance to the P1′ pocket allows for little modification in this region of the inhibitor.

The activity of P1′ analogues of **BB-3497** against the PDF enzyme is consistent with a well defined hydrophobic S1′ pocket. In correlation with the structural information obtained from X-ray data, this pocket is capable of accommodating small alkyl, cycloalkyl and benzylic groups. Variation in the length of the P1′ alkyl chain (Table 2) (**23a–23c**, **23e–23h**) revealed that *n*-butyl was optimal (**BB-3497**). However, the enzyme also accommodated a variety of other substituents. Branching of the alkyl chain (**23i–23k**), addition of a cycloalkyl substituent (**23l–23n**) and the presence of unsaturation or a sulfur atom (**23–23s**) in the P1′ side chain all gave

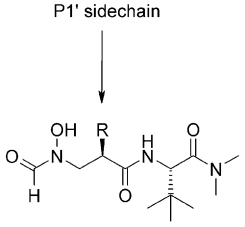
Table 1. PDF enzyme inhibition and antibacterial activity of **BB-3497** and analogues with a modified spacer group



Compd	X	*E. coli* PDF·Ni IC_{50} (nM)	*E. coli* MIC (μM)	*S. capitis* MIC (μM)
BB-3497	**CH_2**	**7**	**12.5**	**25**
4	CH_2CH_2	1000	>200	>200
16	CHMe	600	>200	>200
9a	*n* = 0 (*R*-isomer)	20%@1μM[a]	>200	>200
9b	*n* = 0 (*S*-isomer)	30%@1μM[a]	>200	>200

E coli, *Escherichia colia*; *S. capitis*, *Staphylococcus capitis*.
[a]IC_{50} expressed as percentage inhibition at a concentration of 1 μM.

Table 2. PDF enzyme inhibition and antibacterial activity of **BB-3497** and its P1′ analogues



Compd	P1′ (R)	*E. coli* PDF·Ni IC_{50} (nM)	*E. coli* MIC (μM)	*S. capitis* MIC (μM)
23a	Me	100	>200	>200
23b	Et	70	200	>200
23c	*n*-Pr	50	50	>200
BB-3497	*n*-Bu (*R*-isomer)	7	12.5	25
23d	*n*-Bu (*S*-isomer)	70	>200	>200
23e	*n*-Pentyl	10	25	25
23f	*n*-Hexyl	30	100	200
23g	*n*-Heptyl	40	>200	>200
23h	*n*-Octyl	50	>200	>200
23i	*i*-Pr	50	>200	>200
23j	*i*-Bu	10	100	200
23k	*i*-Pentyl	20	50	200
23l	*c*-Pentylmethyl	8	12.5	12.5
23m	*c*-Pentyl	20	>200	>200
23n	*c*-Hexylmethyl	6	50	25
23p	Allyl	30	50	>200
23q	But-3-enyl	20	25	200
23r	But-2-ynyl	30	50	100
23s	$EtSCH_2$	20	25	>200
23t	Bn	20	25	50
23u	Ph(4-Cl)	500	>200	>200
23v	$(4\text{-MeO})PhCH_2$	200	>200	>200
23w	1-Piperidylmethyl	>1000	>200	>200

A 2-fold difference in PDF·Ni enzyme potency can be regarded as significant when comparing IC_{50} values between compounds.

rise to potent PDF enzyme inhibitors. Incorporation of a benzylic group was acceptable (**23t**) but a substituent on the ring (**23v**) resulted in a 10-fold reduction in PDF inhibition. Introduction of a basic nitrogen (**23w**) and a directly linked aromatic group (**23u**) were detrimental to PDF inhibition.

A limited number of compounds having the (*S*)-configuration at the P1′ centre were assayed and shown to be less potent against the PDF enzyme when compared to the (*R*)-diastereoisomer. This is highlighted in the case of **BB-3497** and **23d**. The data also suggest a modest advantage in activity against Gram-positive organisms is gained by replacing the *n*-butyl in **BB-3497** with a cyclopentylmethyl substituent (**23l**).

Compounds with similar PDF activity often have a markedly different antibacterial profile (e.g., **23m** vs **23t**). These differences may result in part from relative lipophilicities but other factors enabling access to the bacteria and/or susceptibility to efflux mechanisms may be important. Modifications adjacent to the metal binding group and to the *n*-butyl substituent are limited due to the steric requirements for binding the active site metal and size of the hydrophobic S1′ pocket.[5–8] Substituents that closely mimic the methionine of the substrate provide optimal PDF enzyme inhibition. Following this study we have focused on the modification of substituents in the P2′ and P3′ positions to optimise the microbiological profile. Preliminary results from this study are reported in the following communication.[11]

In conclusion, a series of **BB-3497** analogues containing modifications to the spacer group and the P1′ side chain have been synthesised and were tested in the PDF enzyme and whole cell assays. The optimum distance between the metal binding group and the P1′ position on the pseudopeptide backbone of **BB-3497** was found to be an unsubstituted methylene unit. The optimum groups in the P1′ position for PDF enzyme inhibition and antibacterial activity were found to be the *n*-butyl and the cyclopentylmethyl substituent.

Acknowledgements

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