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Structure–Activity Relationships of the Peptide Deformylase Inhibitor BB-3497: Modification of the P2′ and P3′ Side Chains

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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 both the P2′ and P3′ side chains. Enzyme inhibition and antibacterial activity data revealed that a variety of substituents are tolerated at the P2′ and P3′ positions of the inhibitor backbone. The data from this study highlights the potential for modification at the P2′ and P3′ positions to optimise the physicochemical properties.

Bacterial peptide deformylase (PDF) belongs to a subfamily of metalloproteases that catalyse the removal of the N-terminal formyl group from newly synthesised proteins.[1] PDF is essential in prokaryotes and conserved throughout the eubacteria. It is therefore considered an attractive target for developing new antibacterial agents.[2,3]

We reported previously[4] 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,[5] we have reported the structure-based design around the metal binding group. We now wish to report on the systematic modification of the P2′ and P3′ side chains indicated in Figure 1, describing the synthetic approach to this work and the biological data that resulted from the screening process. X-ray crystallographic data[6–10] had revealed that these regions of the PDF enzyme would tolerate a diverse range of substitution and hence it was our intention to explore the effect of introducing a variety of sterically demanding and/or polar substituents at the P2′ and P3′ positions to direct the optimisation of this class of inhibitor.

Chemistry

The preceding paper[11] described the approach used in identifying the optimum distance between the metal binding group and the P1′ sidechain, together with the preferred P1′ side chains for PDF enzyme affinity and antibacterial activity. The *n*-butyl substituent at the P1′ position was optimal for enzyme activity and it was the common intermediate **4**, detailed in Scheme 2, that was used in exploring the effect of sequential modification of substituents at the P2′ and P3′ positions. We were able to make use of a precursor previously reported in the asymmetric route to **BB–3497**[12] to access **4**.

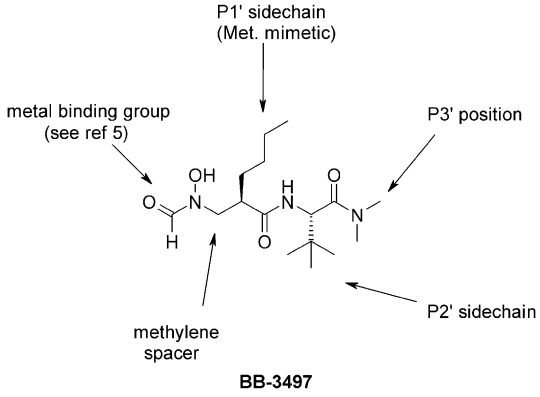

Figure 1. BB-3497 structure–activity relationships.

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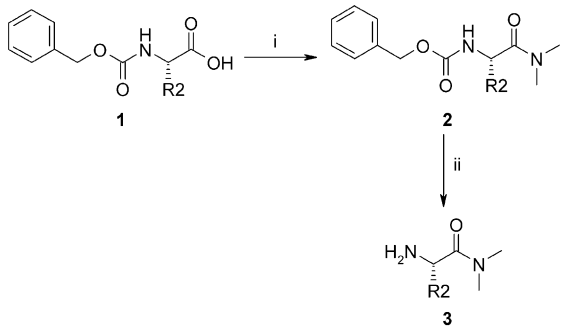
Compounds with modified P2′ substituents were prepared by coupling the appropriate amino acid–NMe_2 derivative **3** (Scheme 1) with 2-[(benzyloxy-formyl-amino)-methyl]-hexanoic acid **4** as detailed in Scheme 2.

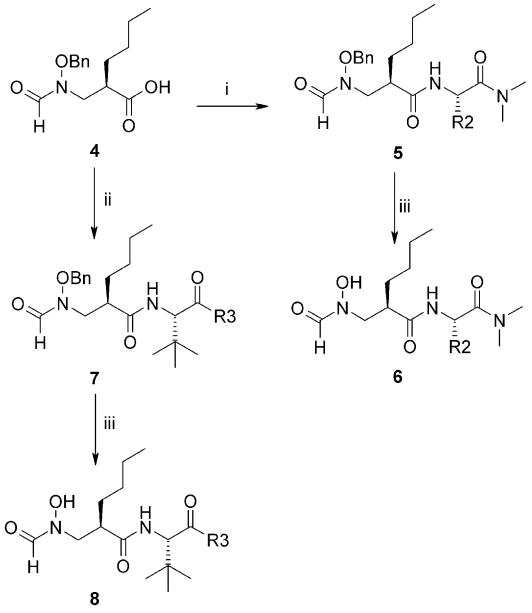
A series of benzyloxycarbonyl (Cbz) protected L-amino acids (**1**) was coupled with dimethylamine, via a parallel synthesis approach using standard peptide coupling conditions (Scheme 1). The unpurified amides (**2**) were then subjected to Pd-catalysed hydrogenation to give the required L-amino acid–NMe_2 (**3**) in quantitative yields.

Standard peptide coupling conditions of **3** with intermediate **4** followed by catalytic hydrogenation, afforded the *N*-formyl hydroxylamine derivative **6** (Scheme 2). A comprehensive list of amino acids investigated is shown in Table 1.

Similarly, for modification of the P3′ substituent (Scheme 3), Cbz-L-*tert*-leucine **9** was treated with a variety of secondary amines as detailed in Table 2. Standard peptide coupling conditions in a parallel

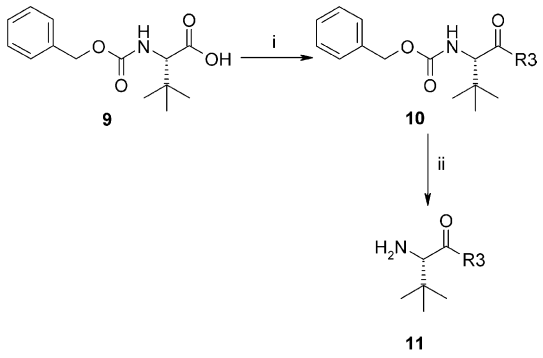


Scheme 1. (i) Me_2NH, EDC, HOAt, DMF, 68–95%; (ii) H_2, Pd/C, MeOH, quant.



Scheme 2. (i) **3a–w**, EDC, HOAt, DMF, 50–75%; (ii) **11a–n**, EDC, HOAt, DMF, 60–80%; (iii) H_2, Pd/C, MeOH, *then* purification by preparative HPLC.

synthesis approach gave the amide compound **10**. Catalytic hydrogenation of the Cbz group provided the P3′ amide analogue **11** without prior purification. Subsequent peptide coupling conditions of **11** to carboxylic acid **4** gave compound **7**, as shown in Scheme 2. Removal of the *O*-benzyl protecting group by Pd-catalysed hydrogenation provided the *N*-formyl hydroxylamines **8a–n**, as detailed in Table 2. Purification of **8a–n** was achieved by preparative HPLC.



Scheme 3. (i) Secondary amine, EDC, HOAt, DMF, 60–85%; (ii) H_2, Pd/C, MeOH, quant.

Results and Discussion

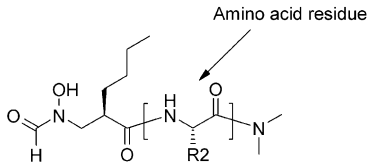
All compounds were screened for activity against the PDF.Ni enzyme and selected Gram-positive and Gram-negative bacterial strains.[13]

The majority of substituents investigated at the P2′ position (**6a–w**) are reasonably well tolerated (Table 1). This correlates with recent X-ray crystallographic data[4] of **BB-3497** suggesting that the P2′ side chain is mostly solvent exposed and makes little contact with the PDF enzyme. Incorporation of certain polar groups, (**6p–q**, **6u**) results in a 30-fold reduction in enzyme activity but the arginine residue (**6r**) offers some potential for enhancing the physicochemical properties of the inhibitors through the introduction of appropriate polar residues. It is, however, noteworthy that the *tert*-leucine of **BB-3497** and the related *S*-methyl penicillamine (**6h**) showed the best in vitro enzyme inhibition and antibacterial profile.

A variety of sterically demanding groups are tolerated at the P3′ position, suggesting little interaction with the enzyme (Table 2). Replacement of the dimethylamido functionality of the P3′ side chain (**BB-3497**) revealed that the methyl groups can be linked to form a five- or six-membered ring (**8d–j**) which can be further fused to a second ring (**8l–m**). Replacement of the amide functionality[14] by an ester (**8a**) or methyl ketone (**8c**) was tolerated but acidic or basic group incorporation caused a modest reduction in PDF enzyme activity (**8a** vs **8b** and **8f** vs **8g**).

In this study, modifications to the P2′ and P3′ side chains have not demonstrated an improved microbiological profile over **BB-3497**. Indeed, compounds

Table 1. PDF enzyme inhibition and antibacterial activity of **BB-3497** and its P2′ analogues

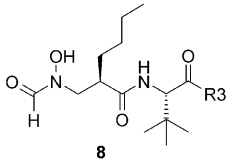


6

Compd	P2′ amino acid (NH-R2-CO)	*E. coli* PDF·Ni IC_{50} (nM)	*E. coli* MIC (μM)	*S. capitis* MIC (μM)
6a	Gly	100	50	>200
6b	Ala	40	25	100
6c	Val	20	25	25
6d	Leu	20	50	50
6e	Cha	30	100	100
6f	Ile	20	25	12.5
BB-3497	*t*-Leu (*S*–isomer)	7	12.5	25
6g	*t*-Leu (*R*-isomer)	40	100	>200
6h	Pen(SMe)	6	25	25
6I	Cys(Bn)	70	200	12.5
6j	Ser	50	100	50
6k	Val(β-OH)	20	12.5	25
6l	Val(β-OMe)	20	25	100
6m	Asp(β-Bn)	100	>200	25
6n	Glu(β-Bn)	20	>200	200
6p	Lys	200	>200	>200
6q	Lys(ε-NMe_2)	300	>200	>200
6r	Arg	20	>200	50
6s	Phe	10	100	50
6t	Phe(4-Cl)	50	>200	100
6u	Tyr	300	>200	>200
6v	L-Tic	80	>200	>200
6w	Pro	400	>200	50

S. capitis, *Staphylococcus capitis*.

Table 2. PDF enzyme inhibition and antibacterial activity of **BB-3497** and its P3′ analogues



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Compd	P3′ (R3)	*E. coli* PDF·Ni IC_{50} (nM)	*E. coli* MIC (μM)	*S. capitis* MIC (μM)
BB-3497	NMe_2	7	12.5	25
8a	OMe	30	50	200
8b	OH	80	>200	>200
8c	Me	20	25	100
8d	Pyrrolidin-1-yl	10	25	25
8e	Morpholin-4-yl	20	25	25
8f	4-Me-piperazin-1-yl	50	25	50
8g	4-Me-piperidin-1-yl	10	100	50
8h	4-Ac-piperidin-1-yl	10	100	25
8i	4-EtO_2C-piperidin-1-yl	10	>200	25
8j	4-Bn-piperidin-1-yl	20	200	6.25
8k	N(Me)*c*-Hexyl	30	>200	100
8l	Decahydroquinoliny-1-yl	40	>200	50
8m	Tetrahydroquinolin-1-yl	9	50	50
8n	N(Bn)CH_2CH_2Ph	90	>200	100

with similar PDF activity often had a markedly different antibacterial profile (e.g., **6k** vs **6n**), highlighting the significant activity profile seen for **BB-3497**. However, the results suggest the potential for modification at the P2′ and P3′ positions to optimise physicochemical properties.[3]

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 P1′ pocket.[10,11] Substituents that closely mimic the methionine of the substrate provide optimal PDF enzyme inhibition. In contrast, a range of substituents and functional groups are tolerated by PDF at the P2′ and P3′ positions. This observation is in line with crystallographic data that suggest these groups are largely exposed to solvent. These structural studies have also highlighted the importance of the backbone amide bonds for enzyme inhibition through hydrogen bonding interactions with the enzyme.

In addition to PDF inhibition, there are clearly a range of factors to consider in identifying an agent with a suitable antibacterial profile. Factors that affect drug distribution such as bacterial membrane permeability, efflux and metabolism are all clearly important in the identification of a compound as a new class of antibiotic.

In conclusion, a series of **BB-3497** analogues with modifications along the pseudopeptide backbone have been synthesised. The results indicate a preference for the *tert*-butyl group at P2′ and for further developing the P3′ group where the P3′ benzylpiperidinyl substituent (**8j**) enhances Gram-positive activity. This has been a focus of our continuing programme and will be reported in due course.

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13. PDF inhibition assays and MICs were performed as described previously.[4]
14. The methyl ester analogue **8a** was prepared from esterification of Cbz-L-*tert*-leucine using diazomethane in dichloromethane. Saponification at the final stage of the synthesis afforded the carboxylic acid **8b**. The ketone compound **8c** was prepared by MeLi addition to the corresponding Weinreb amide.