Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 2003–2005

Preparation of tetra-Boc-protected polymyxin B nonapeptide

Hardwin O'Dowd,* Bum Kim, Peter Margolis, Wen Wang, Charlotte Wu, Sara L. Lopez and Johanne Blais

Pfizer Global Research and Development, Fremont,† CA 94555, USA

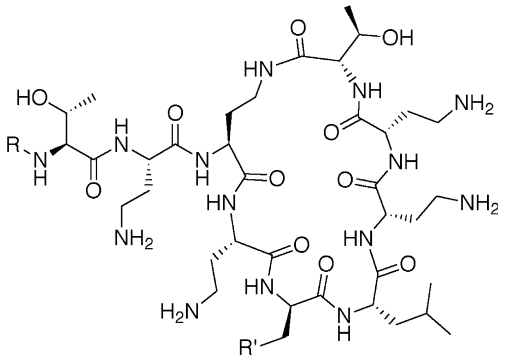
Received 9 April 2006; revised 15 January 2007; accepted 15 January 2007
Available online 18 January 2007

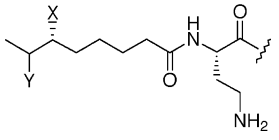
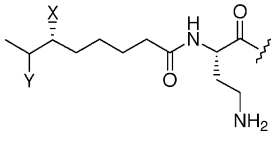
Abstract—A method for the selective tetra-Boc-protection of polymyxin B nonapeptide (PMBN) has been developed. Boc-ON selectively protects the amino side chains of the four diaminobutyric acid (Dab) residues in the presence of the N-terminal free amine.

The polymyxin class of antibiotics, exemplified by polymyxin B_1/B_2 (PMB_1/PMB_2, **1**) and colistin (**2**), is increasingly used to treat nosocomial infections caused by multidrug resistant Gram-negative bacteria.[1] Polymyxins are used clinically as the last therapeutic option due to the adverse effects associated with their administration (e.g., nephrotoxicity, neuromuscular blockade).[2] Polymyxin B (Table 1) acts on the Gram-negative bacterial cell wall, disrupting the permeability of both the outer membrane and the cytoplasmic membrane, resulting in leakage of intracellular components and cell death.[3,4] While possessing only low innate antibacterial activity, polymyxin B nonapeptide (PMBN, **3**) does increase the permeability of the lipopolysaccharide outer membrane,[3] and has been shown to synergize the activity of other antibiotics.[5,6]

Previous reports have identified N-terminal PMBN and colistin nonapeptide derivatives possessing reduced acute toxicity.[7–11] In an effort to expand upon literature SAR, we have initiated a medicinal chemistry program targeting PMBN derivatives, with the goal of the identification of new polymyxin analogs with improved therapeutic index. Literature routes to nonapeptide derivatives have included direct acylation of colistin nonapeptide under buffered conditions,[7–10] and more recently, total synthesis.[11,12] In this Letter, we describe an efficient method for the selective Boc-protection of the basic amino side chains of PMBN. This provides a robust intermediate that allows the use of the orthogonally protected α-*N*-Fmoc-amino acid building blocks for the derivatization of the N-terminal threonine esidue.

Table 1. Polymyxin B and related compounds

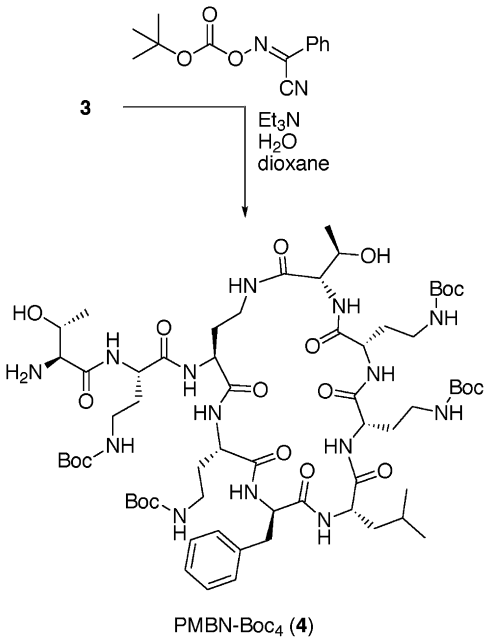


Compound	R	R′
Polymyxin B (**1**) PMB_1 X = Me, Y = H PMB_2 X = H, Y = Me		Ph
Colistin (**2**)		*i*-Pr
Polymyxin B nonapeptide (**3**)	H	Ph
5	Octanoyl	Ph
6	Nonanoyl	Ph
7	Decanoyl	Ph

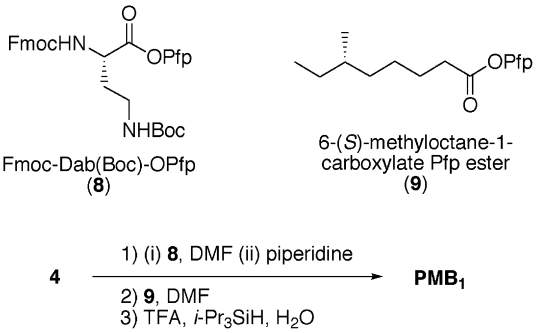
* Corresponding author. E-mail: hardwin@hotmail.com
† Formerly Vicuron Pharmaceuticals, Inc.

0040-4039/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.tetlet.2007.01.071

PMBN is readily prepared from commercial polymyxin B[13] via papain-mediated enzymatic cleavage of the N-terminal fatty acyl group and diaminobutyric acid (Dab) residue.[14] For the subsequent selective Boc-protection, purification of the resulting PMBN was not necessary; it was used crude (maximum 70% pure by weight, cleavage of 12 g of polymyxin B sulfate gave 14 g of crude material **3**). It was recognized that selective protection of the Dab amino groups in the presence of the N-terminal amine could be possible, based on both steric and electronic factors, the Dab amino groups being both less sterically hindered and more electron-rich than the N-terminus. 1-(Boc-oxyimino)-2-phenylacetonitrile[15] (Boc-ON) was discovered to confer the desired selectivity in the protection reaction: treatment of a solution of crude PMBN (approximately 0.05 M) in H_2O–dioxane–Et_3N (1:1:1) with 5 equiv of Boc-ON gave the desired tetra-Boc-protected PMBN (30–40% overall yield from polymyxin B) (Scheme 1). The reaction was complete in less than 20 min at 23 °C; quenching with an excess of methanolic ammonia was necessary to avoid complete conversion to the penta-Boc byproduct. HPLC (UV detector, 220 nm) monitoring of the reaction was somewhat hampered by the dominant chromaphore of the Boc-ON oxime byproduct. A three-step purification protocol was developed as follows: (1) Crude residue (isolated via concentration of quenched reaction mixture) was dissolved in methanol, then filtered. (2) The methanolic solution was added to an excess of diethyl ether; precipitated PMBN–Boc_4 was isolated via filtration (this step serves to remove the oxime byproduct). (3) Crude PMBN–Boc_4 was then subjected to flash column chromatography (silica gel) using Et_3N–MeOH–CH_2Cl_2 (1:13:86) as the mobile phase. The two-step synthetic sequence was reproducible at a multi-gram scale, routinely providing 4–5 g of purified PMBN–Boc_4 from 12 g of polymyxin B sulfate.



Scheme 1. Selective Boc-protection of PMBN.



Scheme 2. Semi-synthesis of polymyxin B_1 from **4**.

Table 2. MIC (μg/mL) data for commercial and synthetic PMB and PMBN derivatives

Compound	*E. coli*	*E. cloacae*	*P. aeruginosa*	*S. aureus*
1 (Commercial)	0.25	0.25	1	>16
PMB_1 (Synthetic)	0.25	0.5	1	>16
3	>16	>16	>16	>16
5	0.03	16	4	>16
6	0.5	16	2	>16
7	1	4	2	16

The identity of the product was confirmed via the Edman degradation reaction. Thus, treatment of **4** with phenylisothiocyanate and acid hydrolysis of the resultant adduct provided polymyxin B octapeptide and the corresponding phenylthiohydantoin threonine. Further confirmation was achieved via the resynthesis of polymyxin B_1 from **4** (Scheme 2).[16]

Conversion of **4** into a series of three *N*-alkanoyl nonapeptide derivatives (**5**–**7**) was achieved simply by acylation followed by TFA-mediated Boc-deprotection. The resulting analogs showed similar trends in activity to those described for the corresponding colistin nonapeptide derivatives. Somewhat striking is the excellent activity of the *N*-octanoyl derivative **5** versus *Escherichia coli*, although it comes at the expense of activity against *Pseudomonas aeruginosa*. The activity versus *Enterobacter cloacae* for the PMBN *N*-acyl derivatives was uniformly less than that of **1**. Weak activity against the Gram-positive pathogen *Staphylococcus aureus* was observed for the *n*-decanoyl derivative **7**, mirroring the Gram-positive activity reported for the longer *n*-alkanoyl derivatives of colistin nonapeptide[8] (Table 2).

In summary, the selective Boc-protection of polymyxin B nonapeptide has been achieved. The resulting tetra-Boc compound is an ideal precursor to N-terminal derivatives of PMBN, which show potential for novel biological activities.

References and notes

1. Li, J.; Nation, R. L.; Milne, R. W.; Turnidge, J. D.; Coulthard, K. *Int. J. Antimicrob. Agents* **2005**, *25*, 11–25.

2. Ouderkirk, J. P.; Nord, J. A.; Turett, G. S.; Kislak, J. W. *Antimicrob. Agents Chemother.* **2003**, *47*, 2659–2662.
3. Dixon, R. A.; Chopra, I. *Antimicrob. Agents Chemother.* **1986**, *29*, 781–788.
4. Storm, D. A.; Rosenthal, K. S.; Swanson, P. E. *Ann. Rev. Biochem.* **1977**, *46*, 723–763.
5. Ofek, I.; Cohen, S.; Rahmani, R.; Kabha, K.; Tamarkin, D.; Herzig, Y.; Rubinstein, E. *Antimicrob. Agents Chemother.* **1994**, *38*, 374–377.
6. Tsubery, H.; Ofek, I.; Cohen, S.; Eisenstein, M.; Fridkin, M. *Mol. Pharmacol.* **2002**, *62*, 1036–1042.
7. Chihara, S.; Tobita, T.; Yahata, M.; Ito, A.; Koyama, Y. *Agric. Biol. Chem.* **1973**, *37*, 2455–2463.
8. Chihara, S.; Ito, A.; Yahata, M.; Tobita, T.; Koyama, Y. *Agric. Biol. Chem.* **1973**, *37*, 2709–2717.
9. Chihara, S.; Ito, A.; Yahata, M.; Tobita, T.; Koyama, Y. *Agric. Biol. Chem.* **1974**, *38*, 521–529.
10. Chihara, S.; Ito, A.; Yahata, M.; Tobita, T.; Koyama, Y. *Agric. Biol. Chem.* **1974**, *38*, 1767–1777.
11. Tsubery, H.; Ofek, I.; Cohen, S.; Fridkin, M. *J. Med. Chem.* **2000**, *43*, 3085–3092.
12. Tsubery, H.; Ofek, I.; Cohen, S.; Fridkin, M. *Peptides* **2001**, *22*, 1675–1681.
13. Commercial polymyxin B is a mixture of polymyxin B_1 and B_2.
14. Danner, R. L.; Joiner, K. A.; Rubin, M.; Patterson, W. H.; Johnson, N.; Ayers, K. M.; Parrillo, J. E. *Antimicrob. Agents Chemother.* **1989**, *33*, 1428–1434.
15. Itoh, M.; Hagiwara, D.; Kamiya, T. *Tetrahedron Lett.* **1975**, *16*, 4393–4394.
16. Synthesis of 6-(*S*)-methyloctane-1-carboxylic acid: Sharma, S. K.; Wu, A. D.; Chandramouli, N.; Fotsch, C.; Kardash, G.; Bair, K. W. *J. Peptide Res.* **1999**, *53*, 501–506.