Peptaibols: models for ion channels

J. K. Chugh and B. A. Wallace[1]
Department of Crystallography, Birkbeck College, University of London, London WC1E 7HX, U.K.

Abstract

Peptaibols are membrane-active polypeptides isolated from fungal sources. They are characterized by the presence of an unusual amino acid, α-aminoisobutyric acid, and a C-terminal hydroxylated amino acid. Peptaibols exhibit antibiotic activity against bacteria and fungi. Their amphipathic nature allows them to self-associate into oligomeric ion-channel assemblies which span the width of lipid bilayer membranes. Over 200 peptaibol sequences have been reported to date, which are compiled in the Peptaibol Database at http://www.cryst.bbk.ac.uk/peptaibol. Alignments of these sequences have been carried out in order to define a series of related subfamilies (SFs) with common sequence features thought to be important for channel formation. Crystal structures determined for a number of peptaibols from the various SFs provide the bases both for modelling of the channel structures and for modelling structures of other members of the same SFs.

Introduction

The peptaibols are a family of antibiotic peptides isolated from soil fungi that exhibit anti-bacterial and anti-fungal properties. They range between five and 20 residues in length. The name peptaibol derives from their chemical composition: peptides containing Aib (α-aminoisobutyric acid or α-methyl alanine) residues and ending in a C-terminal alcohol. They also often contain the residue Iva (isovaleric acid or α-ethyl alanine) and many have a number of imino acids, either proline or hydroxyproline. Aib residues tend to promote formation of helical structures due to the steric constraints imposed by the second methyl group on the Cα atom. All peptaibol structures determined to date are highly helical. The imino acids tend to promote formation of bends or kinks in these structures.

Peptaibols are amphipathic in nature and this property allows many of them to form voltage-dependent ion channels in lipid bilayer membranes [1]. Leakage of cytoplasmic material can occur through such channels, leading to cell death, and this may be the basis of their antibiotic function.

Over 200 peptaibols have been sequenced. Due to the presence of the non-standard amino acids such as Aib and Iva, these sequences are generally not included in most sequence databases. Hence, a database specifically for peptaibols was created and is accessible at http://www.cryst.bbk.ac.uk/peptaibol; this database also includes the sequences as well as the known crystal and NMR structures of family members.

Sequence alignments into subfamilies (SFs)

Alignments of all the sequences in the database have been carried out using ClustalW V1.8 [2] in order to group them into SFs, primarily on the basis of sequence identity, and, to a lesser extent, sequence length. The SFs were further aligned manually in CINEMA V2.1 [3]. In total, there were nine distinguishable SFs (Figure 1). For the purposes of clarity, in the longer SFs (SFs 1–4) only a few representative sequences have been included in Figure 1, which highlights features seen throughout the SFs.

SF1 consists of ≈120 sequences, and is the largest SF, containing over half the peptaibol sequences. This SF is of the 'long' peptaibols, with lengths ranging between 17 and 20 residues. Characteristic of many members of this SF is the presence of a Gln near the middle, often at position 6 or 7. Additional Gln or Glu residues are found towards the C-termini. In many cases, positions 18 and 19 are a Gln-Gln or Glu-Gln pair. In the shorter members of this SF, however, usually only a single Gln residue is present in the C-terminal region. The Glu and Gln residues appear to be located in the pore lumen, important for conductance. In a study where Glu was substituted by Leu in one well-studied member of the SF, alamethicin, channel-forming ability was still observed, so it is not essential that this is a charged residue [4]. However, in another study [5] comparing trichocellins TCA-II and TCB-II, which have, respectively, Gln and Glu at this

Key words: alamethicin, crystal structure, homology modelling, membrane, sequence database.
Abbreviations used: SF, subfamily; Aib, α-aminoisobutyric acid; Iva, isovaleric acid.
[1]To whom correspondence should be addressed (e-mail ubcg25a@mail.cryst.bbk.ac.uk).

position, the molecules with Glu at this position formed channels with longer lifetimes. Prolines are often found in SF1 at positions 13 or 14, where they are proposed to be involved in the mechanism of insertion into membrane bilayers. Prolines in this region create helix-breaking kinks in the structures [6]. There is a Gly at position 11 in ≈80% of the members of the SF. This residue may also have an important role in helix bending, flexibility and insertion. In all SF1 sequences, Aib residues occur with high frequency and along the entire length of the molecules, indicating that these molecules should form predominantly α-helical structures. It has been observed for many membrane proteins [7], and appears also to be true for peptaibols [8], that aromatic amino acids tend to be conserved and located at the bilayer hydrophobic/hydrophilic interfaces. All but a few of the SF1 members have an aromatic amino acid at their C-termini. In the case of the chrysospermins and boletusin [9,10], aromatic residues are present at both termini, Phe at the N-terminus and Trp at the C-terminus. The aromatic residues may aid in stabilization of channels within the membrane.

SF2 and SF3 are the most similar to SF1. SF2 consists of ≈ 30 sequences of peptaibols from six different fungi. They tend to range in size from 14 to 16 residues, and thus although also classified as 'long' peptaibols, are shorter than members of SF1. In general, they have aromatic (Phe) residues at both their N- and C-termini. All have a frequent occurrence of Aib residues and highly conserved Gly and Gln residues in positions 6 and 11 respectively. SF2 is different from SF1 in that its members have imino acids, either Pro or Hyp, that are conserved in both positions 10 and 13. In the antiamoebins, an additional imino acid is found in the C-terminal portion of the molecule.

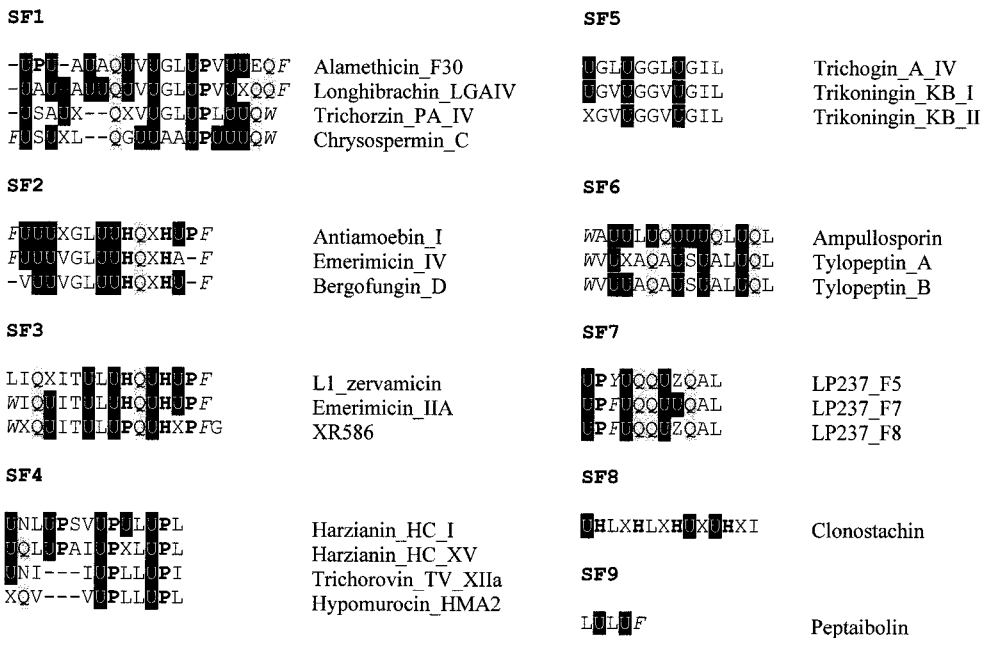
In the closely related SF3, which contains 15 sequences from three different fungal sources, imino acids are found in positions 10, 13 and 15. Two differences between SF3 and SF2 are that in SF3 position 6 is a conserved Thr and, for the most part, in SF3 no Gly residues are present [11]. Like many members of SF1, members of SF3 tend to have two Glns, although in this case the conserved positions are 3 and 11. SFs 2 and 3 are similar in that they tend to have aromatic residues at both the N- and C-termini, unlike SF1, which tends to have them only at the C-terminus. This suggests that the slightly shorter peptaibols may require an extra aromatic amino acid to provide anchorage in the membrane.

SF4 is very different from the other families. This SF consists of peptaibols of either 11 or 14 residues and is made up of ≈50 sequences. In position 2, they have a conserved Gln or Asn residue. In the members with 14 residues, three

Figure 1

Sequence alignments of representative members of the various peptaibol SFs

U = Aib, H = Hyp, X = Iva, Z = ethyl-norvaline. In order to indicate similarities, the following key is used: Aib residues are black, Gln are shaded grey, imino acids are bold and aromatic amino acids are in italics.



Pro residues are present (positions 5, 9 and 13). In the 11-residue members, two Pro positions (equivalent to 9 and 13) are conserved. No aromatics are present in this SF, nor are any charged residues; however, these peptaibols still form channels.

SF5, a group of 'short' peptaibols, at present consists of only five members isolated from three different fungal sources. The sequences consist of either seven or 11 residues. In this SF no Pro, Gln or charged residues are present, possibly indicating that they employ different insertion and conductance mechanisms. Members of SF5 are Gly-rich [12] and it has been suggested that the glycines may line the channel lumen, permitting ions to complex with the polypeptide backbone carbonyl groups, instead of with side chains.

The last SFs, 6, 7, 8 and 9, are extremely small families; SF6 and SF7 have only three sequences each. The members of SF6 come from two fungal sources [13,14], whereas in SF7 all members are homologues from a single fungal source. Members of SF6 are different from those in other SFs in that the hydrophobic aromatic residue Trp is conserved in position 1. All SF6 sequences are 15 residues long, hence they are considered to be long peptaibols. When compared with the other long peptaibols, they tend to have a higher Aib content. All the sequences in this SF have a conserved Gln in position 14 and a conserved Leu at the C-terminal position 15. Pro or Hyp residues are rare.

SF7 consists of the LP237s [15], homologues with a conserved Leu as the C-terminal residue. Out of 11 residues, there are three conserved Gln residues in positions 5, 6 and 9. A conserved Pro is found in position 2, and, unusually for the middle of a sequence, an aromatic residue in position 3. Due to the frequency of Gln and the presence of Pro residues near the N-termini and also because two members of the SF have an unusual amino acid, ethyl-norvaline, there was difficulty in aligning these sequences to other peptaibol sequences.

SFs 8 and 9 contain only single sequences and are so different they could not be put into any of the other SFs using the criterion of $\approx 50\%$ (or higher) identity established for the other SFs. SF8 is clonostachin [16], which has 14 residues, of which just three are Aib. It has four Hyp residues, more than any other peptaibol sequence. No Gln, charged residues or aromatics are present. As a result, clonostachin is an extremely hydrophobic molecule. The last SF, SF9, contains a single sequence, peptaibolin [17], with only five residues. Being the shortest peptaibol reported and having no Pro, Gln or any charged residues, no mechanism for insertion into the membrane has yet been postulated for it, nor has any evidence of channel-forming activity been obtained.

Structure and channel formation by the peptaibol SFs

The high Aib content of peptaibols means that they form helical structures. Only crystal structures of monomeric forms have been determined thus far, but based on these, models have been developed for multimeric transmembrane channels. Their amphipathic nature should allow them to self-associate, and it has been proposed that they form helical bundles, with hydrophobic exteriors in contact with the lipid fatty acid chains, and hydrophilic water-filled interiors.

Crystal structures of representative members of SF1 (alamethicin) [6], SF2 (antiamoebin I) [18,19], SF3 (Leu^1-zervamicin) [20] and SF5 (trichogin-A_IV) [12] have all been solved; these structures permit comparisons between the different SF types. Alamethicin forms a nearly straight and mostly α-helical structure (Figure 2, left-hand structure). Alamethicin molecules are 34 Å long, sufficient to span lipid bilayers, and conductance studies indicate that they are capable of forming channels in a wide variety of lipid molecules. The side chains of Gln-7, Glu-18 and Gln-19 are all located on the same face of the helix, and are likely to form part of the lumen of the channel. The slight bend created by Pro-14, along with the missing hydrogens on the imino nitrogens that result in the absence of a backbone hydrogen bond, allows the carbonyl oxygens of Aib-10 and Gly-11 to also form part of the polar face. It has been proposed that Pro-14 is key for the insertion of alamethicin into the membrane as it forms a bend point between two helical segments [6]. Since helices have overall dipole moments along the direction of their helical axes, it was proposed that the N-terminal helix would insert first into the membrane, leaving Pro-14 on the bilayer edge with the C-terminal helix lying along the membrane surface. Upon application of a voltage, the C-terminus would re-orient itself and insert fully into the membrane; then a number of such helices would associate to form the channel (Figure 3a). This model for insertion was named the 'barrel-stave model' [21]. Alamethicin channels can apparently be composed of between 6 and 12 monomers, but octamers have been inferred to be the most stable conducting forms [6].

The crystal structures of antiamoebin (Figure 2, right-hand structure) and Leu[1]-zervamicin (Figure 2, central structure) [20] strongly resemble each other. Both structures are helical and because of the presence of three Hyp/Pro residues they have a distinct bend in the middle. Both are shorter than alamethicin, with antiamoebin being the shortest due to its larger bend angle (48–56° versus 30–45° for zervamicin). The mechanism of channel formation proposed for antiamoebin is somewhat different from that of alamethicin. Antiamoebin can act as either a carrier or channel and only forms conducting channels in very specific types of lipids [18,22], perhaps as a consequence of the mismatch in length between the peptaibol and lipid molecules. Channels appear to be octamers. Zervamicin can form channels of longer mean channel lifetimes than antiamoebin and in a wider range of lipids, suggesting a relationship between channel stability and length of molecule.

Trichogin-AI_V, from one of the short SFs, is only half the length of alamethicin (16 Å), al-

Figure 2

Comparison of the crystal structures of (left) alamethicin [6], (centre) zervamicin [20] and (right) antiamoebin [18]

Pro residues are indicated in black and Aib residues are in dark grey. The polypeptide backbones are drawn as helical 'worms'. It can be seen that from left to right the structures have an increasing bend angle.

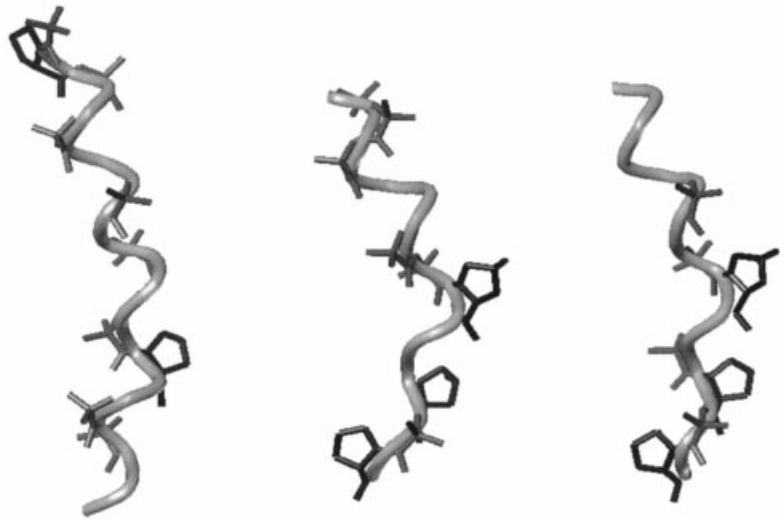
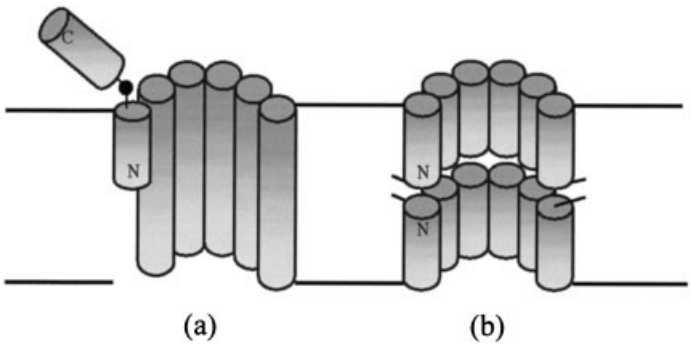


Figure 3

Models for the insertion of (a) long and (b) short peptaibols into membranes

The long peptaibols associate with one another to form channels that span the width of the lipid bilayer. (**a**) One molecule of the bundle shows a proline (represented as a black ball) forming a kink in the structure between the N-terminal and C-terminal helices. After application of a voltage, the entire molecule inserts into the membrane, as shown by the other molecules. (**b**) The N-terminus-to-N-terminus association of two short monomers in the centre of the bilayer is shown.



though it too is helical [12]. It has been proposed (Figure 3b) that the short peptaibols span only half of the lipid bilayer and associate N-terminus to N-terminus in the centre of the membrane. Trichogin does not have a proline-induced bend in the middle, thus the ways it inserts and forms channels are likely to be different from those of the long peptaibols. A 'carpet mechanism' has been proposed for its insertion mechanism, whereby when the local concentration on the surface of the membrane becomes high, the membrane would become disrupted, allowing the peptaibol to enter and form channels in the membrane [23]. In forming a channel, two peptaibols from two different surfaces would form a transmembrane dimer and the octanoyl groups that are present on the N-terminus would promote interactions in the bilayer interior. The importance of the hydrophobic N-terminal group in short peptaibol channel formation was confirmed by studies on harzianin-HB_I, in which there was N-terminal acetylation instead of an acyl chain. Experiments with *Staphylococcus aureus* and *Escherichia coli* indicated that this peptaibol had no membrane-modifying activity [24]. In other studies where different lengths of acyl chains (ranging from two to eight carbons) were added to the 10-residue trikoningin-KB_II, the analogues with shorter acyl chains showed reduced membrane-modifying activity [25].

Functions of peptaibol SFs

Peptaibols exhibit a range of antibiotic functions against different target organisms, although there does not appear to be a clear correlation between SF type and antibiotic activity or target. Some peptaibols show activity against Gram-negative bacteria, some against Gram-positive and some against both; others have activity against fungi. In a number of cases, activity in mammalian cells and/or against viruses has also been demonstrated. The most widely studied member of SF1, alamethicin, has reported activities including channel-formation in bovine adrenal chromaffin cells and transport of Ca^{2+}, Mn^{2+} and Ni^{2+} ions [26,27], along with the release of catecholamines from the adrenal glands of cats [28]. Both alamethicin and SF3 member zervamicin can lyse human erythrocytes; however, SF2 member antiamoebin is not able to do so [29]. Alamethicin is also reported to induce metabolic activity in bovine aorta endothelial cells [26]. The trichokindins and the trichosporins appear to have similar activities to alamethicin in catecholamine secretion [30,31]. Alamethicin, the hypelcins and the trichosporins have all been reported to cause an increase in rat liver respiration [32]. Other SF1 members whose functional activities have been investigated include the chrysospermins, which were reported to have anti-bacterial and anti-fungal activities, and induce pigment formation in the *Phoma destructiva* fungus. They and the saturnisporins have weak activity against *S. aureus* [33]. The trichorzins exhibit activity against both *S. aureus* and fungi [34]. Chrysospermins B and D and the peptaivirins A and B inhibit tobacco mosaic virus [35,36]. The two chrysospermins also showed cytotoxic effects on some cancer cells, with chrysospermin D being more effective than B, thus implicating position 5, which is Aib in chrysospermin B and an Iva in D [36]. Other long peptaibols such as aibellin have been shown to increase rumen fermentation [37]. Although the SF1 members have high (> 80–$90\,\%$) sequence identity, it is clear that the subtle sequence differences produce varying activities.

In SF2, antiamoebin [38] has been described as an anti-amoebic agent. In SF3, XR586 [11] has been shown to have anti-bacterial activity. Other SF3 members such as heptaibin [39] have been reported to have a wide range of anti-bacterial and anti-fungal activities. For shorter peptaibols, fewer functional studies have been reported. In SF5, the trikoningins have activity against *S. aureus* [25].

In SF6, the tylopeptins exhibited activity against Gram-positive bacteria but no activity against pathogenic fungi or Gram-negative bacteria [14], and ampullosporin was reported to have activity as a neuroleptic in mice [13]. Clonostachin from SF8 has been reported to induce ADP-dependent human platelet aggregation [16]. The only member of SF9, peptaibolin, showed moderate activity against some Gram-positive bacteria [17].

In summary, because not all SFs have been tested for each type of activity, direct comparisons cannot be made, and it is not possible to make any strong correlations between functions and different SFs. However, virtually all peptaibols have some membrane-modifying activity.

Common structural features in the peptaibols and ion-channel proteins

The relatively small peptaibol molecules discussed in this paper are finding use as models to aid in our understanding of complex membrane-transport functions. They exhibit a number of features in

common with larger protein channels, such as the potassium and sodium channels [8]. The nature of their lumen, formed from carbonyl groups of the polypeptide backbone or polar side chains, their assembly into multimers to form pores of the correct dimensions for specific ion transport and the presence of aromatic amino acids that act to stabilize the structures within membranes, are all common features exhibited by peptaibols and larger channels. As a result, these relatively simple molecules may be valuable as model systems, providing insight into the complex processes involved in transport across membranes. Peptaibols are particularly good systems for computational simulations of ion binding and translocations due to their small size [40,41], and the wide range of naturally occurring peptaibol sequences provides a wealth of information on functional consequences of structural changes.

J.K.C. was the recipient of a BBSRC studentship. This work was supported, in part, by the BBSRC Bloomsbury Centre for Structural Biology.

References

1 Mueller, P. and Rudin, D. O. (1968) Nature (London) **217**, 713–719
2 Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) Nucleic Acids Res. **22**, 4673–4680
3 Parry-Smith, D. J., Payne, A. W. R., Michie, A. D. and Attwood, T. K. (1997) Gene **211**, GC45–GC56
4 Molle, G., Duclohier, H., Julien, S. and Spach, G. (1991) Biochim. Biophys. Acta **1064**, 365–369
5 Wada, S., Iida, A., Asami, K., Tachikawa, E. and Fujita, T. (1997) Biochim. Biophys. Acta **1325**, 209–214
6 Fox, R. O. and Richards, F. M. (1982) Nature (London) **300**, 325–330
7 Wallace, B. A. and Janes, R. W. (1999) Adv. Exp. Med. Biol. **467**, 789–799
8 Wallace, B. A. (2000) Bioessays **22**, 227–234
9 Dornberger, K., Ihn, W., Ritzau M., Grafe, U., Schlegel, B., Fleck, W. F. and Metzger, J. W. (1995) J. Antibiot. **48**, 977–989
10 Lee, S. J., Yeo, W. H., Yun, B. S. and Yoo, I. D. (1999) J. Pept. Sci. **5**, 374–378
11 Sharman, G. J., Try, A. C., Williams, D. H., Ainsworth, A. M., Beneyto, R., Gibson, T. M., McNicholas, C., Renno, D. V., Robinson, N., Wood, K. A. and Wrigley, S. K. (1996) Biochem. J. **320**, 723–728
12 Toniolo, C., Peggion, E., Crisma, M., Formaggio, F., Shui, X. Q. and Eggleston, D. S. E. (1994) Nat. Struct. Biol. **1**, 908–914
13 Ritzau, M., Heinze, S., Dornberger, K., Berg, A., Fleck, W., Schlegel, B., Hartl, A. and Gräfe, U. (1997) J. Antibiot. **50**, 722–728
14 Lee, S. J., Yun, B. S., Cho, D. H. and Yoo, I. D. (1999) J. Antibiot. **52**, 998–1006
15 Tsantrizos, Y. S., Pischos, S. and Sauriol, F. (1996) J. Org. Chem. **61**, 2118–2121
16 Chikanishi, T., Hasumi, K., Harada, T., Kawaski, N. and Endo, A. (1997) J. Antibiot. **50**, 105–110
17 Hulsman, H., Heinze, S., Ritzau, M., Schlegel, B. and Gräfe, U. (1998) J. Antibiot. **51**, 1055–1058
18 Snook, C. F., Woolley, G. A., Pattabhi, V., Wood, S. P., Blundell, T. L. and Wallace, B. A. (1998) Structure **6**, 783–792
19 Karle, I. L., Perozzo, M. A., Mishra, V. K. and Balaram, P. (1998) Proc. Natl. Acad. Sci. U.S.A. **95**, 5501–5504
20 Karle, I. L., Flippen-Anderson, J. L., Agarwalla, S. and Balaram, P. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 5307–5311
21 Boheim, G. (1974) J. Membr. Biol. **19**, 277–303
22 Duclohier, H., Snook, C. F. and Wallace, B. A. (1998) Biochim. Biophys. Acta **1415**, 255–260
23 Monaco, V., Formaggio, F., Crisma, M., Toniolo, C., Hanson P. and Millhauser, G. L. (1999) Biopolymers **50**, 239–253
24 Augeven-Bour, I., Rebuffat, S., Auvin, C., Goulard, C., Prigent, Y. and Bodo, B. (1997) J. Chem. Soc. (Perkin Trans.) **10**, 1587–1594
25 Piazza, C., Formaggio, F., Crisma, M., Toniolo, C., Kamphuis, J., Kaptein, R. and Broxterman, Q. B. (1999) J. Pept. Sci. **5**, 96–102
26 Dathe, M., Kaduk, C., Tachikawa, E., Melzig, M. F., Wenschuh, H. and Bienert, M. (1998) Biochim. Biophys. Acta **1370**, 175–183
27 Fonteriz, R. I., Lopez, M. G., Garcia-Sancho, J. and Garcia, A. G. (1991) FEBS Lett. **283**, 89–92
28 Artalejo, A. R., Montiel, C., Sanchez-Garcia, P., Uceda, G., Guantes, J. M. and Garcia, A. G. (1990) Biochem. Biophys. Res. Commun. **169**, 1204–1210
29 Bruckner, H., Graf, H. and Bokel, M. (1984) Experientia **40**, 1189–1197
30 Iida, A., Sanekata, M., Fujita, T., Tanaka, H., Enoki, A., Fuse, G., Kanai, M, Rudewicz, P. J. and Tachikawa, E. (1994) Chem. Pharm. Bull. **42**, 1070–1075
31 Nagaoka, Y., Iida, A., Kambara, T., Tachikawa, E., Asami, K. and Fujita, T. (1995) Biol. Pharm. Bull. **18**, 640–642
32 Okuda, M., Iida, A., Uesato, S., Nagaoka, Y., Fujita, T., Takaishi, Y. and Terada, H. (1994) Biol. Pharm. Bull. **17**, 482–485
33 Rebuffat, S., Conraux, L., Massias, M., Auvin-Guette, C. and Bodo, B. (1993) Int. J. Pept. Protein Res. **41**, 74–84
34 Goulard, C., Hlimi, S., Rebuffat, S. and Bodo, B. (1995) J. Antibiot. **48**, 1248–1253
35 Yun, B. S., Yoo, I. D., Kim, Y. H., Kim, Y. S., Lee, S. J., Kim, K. S. and Yeo, W. H. (2000) Tetrahedron Lett. **41**, 1429–1431
36 Kim, Y. H., Yeo, W. H., Kim, Y. S., Chae, S. Y. and Kim, K. S. (2000) J. Microbiol. Biotechnol. **10**, 522–528
37 Kumazawa, S., Kanda, M., Aoyama, H., Utagawa, M., Kondo, J., Sakamoto, S., Ohtani, H., Mikawa, T., Chiga, I. and Hayase, T. (1994) J. Antibiot. **47**, 1136–1144
38 Thirumalachar, M. J. (1968) Hindustan Antibiot. Bull. **10**, 287–289
39 Ishiyama, D., Satou, T., Senda, H., Fujimaki, T., Honda, R. and Kanazawa, S. (2000) J. Antibiot. **53**, 728–732
40 La Rocca, P., Biggin, P. C., Tieleman, D. P. and Sansom, M. S. P. (1999) Biochim. Biophys. Acta **1462**, 185–200
41 Biggin, P. C. and Sansom, M. S. P. (1996) Biophys. Chem. **60**, 99–110

Received 20 February 2001