Post-translational Amino Acid Isomerization

A FUNCTIONALLY IMPORTANT D-AMINO ACID IN AN EXCITATORY PEPTIDE*

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The post-translational modification of an L- to a D-amino acid has been documented in relatively few gene products, mostly in small peptides under 10 amino acids in length. In this report, we demonstrate that a 46-amino acid polypeptide toxin has one D-phenylalanine at position 44, and that the epimerization from an L-Phe to a D-Phe has a dramatic effect on the excitatory effects of the peptide. In one electrophysiological assay carried out, the D-Phe-containing peptide was extremely potent, whereas the unmodified polypeptide had no biological activity, demonstrating that the chirality of the posttranslationally modified amino acid is functionally significant. The peptide toxin analyzed, r11a, belongs to the I-gene superfamily of conotoxins that has four disulfide cross-links. The D-Phe in r11a is at the third amino acid from the C terminus, the same relative position from the C-terminal end as the D-amino acid in ω-agatoxin TK from a spider, an unrelated peptide. Thus, although post-translational amino acid isomerization appears to have no strong specificity for the chemical nature of the amino acid side chain, the few peptides where this modification has been established suggest that there may be favored positions near the N or C terminus that are preferential sites for isomerization to a D-amino acid.

A remarkable post-translational modification of polypeptides synthesized on ribosomes is the conversion of a standard L-amino acid into the corresponding D-isomer (1, 2). Most cases of post-translational isomerization to D-amino acids have been documented in small peptides from vertebrate, arthropod, and molluscan systems. This post-translational modification does not appear to be specific for a particular amino acid; a variety of different amino acids have been post-translationally converted to the D-form. The diverse biological systems in which D-amino acid-containing gene products have been characterized, and the apparently unrelated sequence contexts in which these post-translationally modified amino acids occur raises the question of whether such a post-translational modification can be predicted.

A difficulty in critically assessing the natural distribution of the post-translational isomerization of amino acids is that the larger the gene product, the more difficult it becomes to ascertain the presence of a modified amino acid. In small peptides, the effect of converting a single L- to a D-amino acid can cause a significant difference in chromatographic and other properties; however, as the polypeptide gets larger, converting one amino acid residue to the corresponding D-isomer results in more subtle global changes. Furthermore, in contrast to all other post-translational modifications, isomerization to a D-amino acid is not detectable by any of the standard techniques used in proteomics, such as mass spectrometry. In order to be able to predict when the post-translational conversion to a D-amino acid might occur, the identification of more bona fide cases of natural polypeptides having a D-amino acid is required.

The first larger peptidic gene product in which a D-amino acid was identified was ω-agatoxin TK from the funnel spider Agelenopsis aperta: a single D-serine was identified toward the C-terminal end (3). The only other larger peptide with a D-amino acid, also from an arthropod system, was the crustacean hyperglycemic hormone, where the amino acid modified is toward the N-terminal end (4–6). There is no similarity at all that can be detected between the sequence context of the post-translational isomerization in these two cases.

Among peptides that have been modified from different sources such as frog skin, molluscan nervous tissue, or platypus venom, the D-amino acid is most often located at the second position from the N terminus (7–14). However, in the contrypphans, a group of octapeptides from the venom of Conus, the D-amino acid is found at the fourth amino acid residue (15–17). In both the frog peptides and in the contrypphants, the position within the peptide seems to be the dominant determinant of whether a change in chirality from an L-amino acid to a D-amino acid will occur, rather than the type of amino acid at that position.

In this work, we describe the presence of a D-amino acid in a member of a recently characterized group of conotoxins, the I-superfamily (18). This conotoxin, r11a, is only the third example found of a larger polypeptideic gene product, which has definitively been shown to have undergone this unusual post-translational modification. We have synthesized both the L- and the D-amino acid-containing isomers of conotoxin r11a, and demonstrate that the presence of the modified amino acid causes a marked change in biological activity.

EXPERIMENTAL PROCEDURES

Purification of Conotoxin r11a from Venom—Crude venom extract was prepared as described earlier (15). The extract was loaded onto a Vydac C18 semipreparative HPLC1 column and eluted at 5 ml/min with

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1 The abbreviations used are: HPLC, high performance liquid chromatography; MTBE, methyl-tert-butyl ether; MALDI, matrix-assisted laser desorption ionization; HBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIEA, N,N-diisopropyl-ethylamine; i.c., intracranially; MS, mass spectrometry.
a gradient of solvent A (0.1% trifluoroacetic acid) and solvent B₉₀
(0.05% trifluoroacetic acid in 90% acetonitrile). Further purification was done on a Vydac C₁₈ analytic column at a flow rate of 1 ml/min.

Matrix-assisted laser desorption ionization (MALDI) mass spectra were obtained using a Voyager DE STR mass spectrometer, courtesy of the Mass Spectrometry and Proteomic Core Facility, University of Utah. Alternatively, MALDI spectra were obtained using a Bruker REFLEX time-of-flight mass spectrometer (Bruker Daltonics) fitted with a gridless reflectron, an N2 laser, and a 100 MHz digitizer, courtesy of the Salk Institute for Biological Studies (La Jolla, CA).

**Digestion with Chymotrypsin—**Digestion products were analyzed by MALDI mass spectrometry. Additionally, peptide fragments were digested with endoproteinase Asp-N as described under Peptide Synthesis—. Both natural and synthetic conotoxin r11a were digested with endoproteinase Asp-N (Roche Applied Science) to generate fragments. Two nanomoles of the reduced and alkylated peptide were dissolved in 40 μl of 50% acetonitrile in 0.1% trifluoroacetic acid, and reduced by addition of 10 μl of reduction buffer (0.5 M Tris-acetate, pH 8.0, containing 5 mM EDTA and 100 mM dithiothreitol) and incubation at 65 °C for 30 min. The peptide was then alkylated with 40 μl of 0.5 M iodoacetic acid in 0.5 M Tris-acetate, pH 8.0, containing 5 mM EDTA in the dark at room temperature for 30 min. The reaction mixture was applied to a Vydac C₁₈ analytical HPLC column and eluted with 30% solvent B for 15 min followed by a gradient of 30–55% solvent B for 25 min.

**Digestion with Endoproteinase Asp-N—**Both natural and synthetic conotoxin r11a were digested with endoproteinase Asp-N (Roche Applied Science) to generate fragments. Two nanomoles of the reduced and alkylated peptide were dissolved in 40 μl of 50% acetonitrile in 0.1% trifluoroacetic acid, and reduced by addition of 10 μl of reduction buffer (0.5 M Tris-acetate, pH 8.0, containing 5 mM EDTA and 100 mM dithiothreitol) and incubation at 65 °C for 30 min. The peptide was then alkylated with 40 μl of 0.5 M iodoacetic acid in 0.5 M Tris-acetate, pH 8.0, containing 5 mM EDTA in the dark at room temperature for 30 min. The reaction mixture was applied to a Vydac C₁₈ analytical HPLC column and eluted with 30% solvent B for 15 min followed by a gradient of 30–55% solvent B for 25 min.

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The linear peptide with all L-amino acids was chemically synthesized and folded, and the resultant synthetic peptide was shown to elicit excitatory biological activity. However, a biochemical characterization of folded synthetic peptide revealed that it did not co-elute with the natural peptide. When the natural peptide was reduced, the linear synthetic peptide and the linear natural peptide also did not co-elute. However, the mass value of the synthetic material was identical to that of the natural peptide. A preliminary functional characterization indicated that the synthetic peptide, though biologically active, was not as potent as natural r11a. Together, these initial data raised the possibility that a d-amino acid residue might be present in the natural conotoxin r11a.

### RESULTS

**Non-identity of Synthetic Conotoxin (All L Version) and Natural Conotoxin r11a: Preliminary Evidence Suggesting the Presence of a d-Amino Acid—**The excitatory conotoxin r11a which belongs to the I-superfamily is a relatively large conotoxin (46 amino acids) with four disulfide bonds, purified from the venom of the piscivorous species *Conus radiatus*. The purification of the natural conotoxin used in the studies described below was carried out by a modification of the method described previously (18). *C. radiatus* venom has at least 18 different I-superfamily peptides; all that have been purified from venom are excitatory. A major challenge in purification is the separation of one isoform from another. As shown in Fig. 1, peptide r11a was purified to homogeneity, but this required four reverse-phase HPLC runs to separate the peptide from contaminating isoforms.

In order to further explore this possibility, both the reduced and alkylated synthetic (all L version) and natural peptides were digested with endoproteinase Asp-N as described under "Experimental Procedures." After digestion with the endoproteinase, the products were analyzed by HPLC. Three digestion products were identified by mass spectrometry. These were the predicted end digestion products of endoproteinase Asp-N (Fig. 2A): an N-terminal heptapeptide (P2), a nonapeptide from residues 8 to 16 (P1) and a large C-terminal fragment from residues 17 to 46 (P3). The two smaller peptides from synthetic and natural r11a were identical in their elution properties on HPLC, but there was a small shift between the synthetic and
natural peptide for P3, the large C-terminal product. This indicated that any D-amino acid(s) were likely to be between amino acids 17 and 46. The reduced and alkylated natural and synthetic linear peptides yielded different products upon digestion with chymotrypsin (Fig. 2B). For the synthetic peptide, four different digestion products were obtained. A combination of mass spectrometry and Edman sequencing was used to identify the cleavage products. This analysis revealed that chymotrypsin cleavage after Trp33 and Phe44 had occurred on the synthetic peptide, but that the digestion at these loci was incomplete. Thus, two of the predicted digestion products, the fragments from amino acids 1 to 33 (P4) and 34 to 44 (P5) were obtained, as well as two incomplete digestion products (1–44 (P7) and 34–46 (P6)). However, when a similar digestion of the natural peptide was carried out, a strikingly different pattern was obtained. No incomplete digestion products (1–44 (P7) and 34–46 (P6)) were obtained. A combination of mass spectrometry and Edman sequencing was used to identify the cleavage products. This analysis revealed that chymotrypsin cleavage after Trp33 and Phe44 had occurred on the natural peptide, but that the digestion at these loci was incomplete. Thus, two of the predicted digestion products, the fragments from amino acids 1 to 33 (P4) and 34 to 44 (P5) were obtained, as well as two incomplete digestion products (1–44 (P7) and 34–46 (P6)).

We reasoned that if residue 44 were in fact a D-amino acid, the failure of chymotrypsin to cleave after Phe44 in the natural peptide would be explained. Thus, together, the data led us to the specific working hypothesis that natural conotoxin r11a differs in having a D-phenylalanine residue at position 44, instead of the L-Phe in the synthetic peptide.

Direct Verification of the Presence of a D-Amino Acid in r11a by Chemical Synthesis—The hypothesis that r11a contains a D-phenylalanine residue at position 44 was directly tested by chemical synthesis. Peptide P6 with amino acids from 34 to 46 was chemically synthesized with both L-Phe44 and D-Phe44; when these synthetic peptides were chromatographed with the corresponding chymotrypsin fragment from natural r11a, only the D-Phe44 peptide co-eluted, and the L-Phe44 peptide was resolved from the natural 34–46 fragment. These results led us to synthesize the entire peptide r11a as described under “Experimental Procedures.” An all L version and a D-Phe44 version of the r11a primary sequence were synthesized, and both peptides were folded. As is shown in Fig. 3, only the D-Phe44-containing conotoxin r11a co-eluted with the natural peptide. This provides direct biochemical evidence that Phe44 is post-translationally modified from an L- to a D-amino acid in the natural conotoxin. Thus, the correct amino acid sequence of the conotoxin r11a is: GOSFKADJEKCYHADCCNCCLSLGICAOStNWILPGCTSSFFKI, where F is D-phenylalanine. We refer to the D-Phe44 version of the peptide simply as r11a and the all L version as [L-Phe44]r11a.

Biological Activity of Synthetic Peptides r11a and [L-Phe44]r11a—A functional comparison of the folded r11a analogs with different chirality at position 44 was carried out both by an electrophysiologica characterization with an in vitro nerve-muscle preparation from frog, as well as by using an in vivo behavioral assay on mice. Unlike either natural or synthetic r11a, both of which induce repetitive activity in the amphibian nerve-muscle preparation at concentrations as low as 0.1 μM (see below), no repetitive activity was seen with [L-Phe44]r11a when tested at concentrations up to 25 μM. Thus,
minutes to appear (see Fig. 4 for examples). At 100 nM, the mean (±S.D.) induction time in three trials with each peptide was 3.8 ± 0.8 min for the natural peptide and 4.9 ± 2.2 min for the synthetic peptide. Thus, within experimental error, the peptides have the same on-rate. 2) Off-rate. The repetitive activity induced by both peptides persisted for >18 h following their washout. Thus, the effect of both peptides is relatively irreversible. 3) When the peptide-induced activities are simultaneously recorded in nerve and muscle, it was observed that each action potential in the muscle is preceded by an action potential in the nerve. This was originally reported for the native peptide (18) and is also seen with the synthetic peptide (Fig. 5). This indicates that with both natural and synthetic peptides, repetitive activity is primarily induced in the motor nerve and only secondarily in the muscle. That is, the peptide induces repetitive activity in the motor nerve, and this activity is conveyed to the muscle via synaptic transmission. As would be expected, when synaptic transmission is blocked by exposing the preparation to 5 μM d-tubocurare, an acetylcholine receptor-antagonist, repetitive activity in the muscle, but not in the nerve, is blocked (not illustrated).

**DISCUSSION**

The studies above have demonstrated the presence of an unusual post-translationally modified amino acid, D-phenylalanine, at amino acids 44 of r11a, a conotoxin with eight Cys residues and 46 amino acids. This is the first multiple disulfide-bonded conotoxin shown to contain a D-amino acid. The post-translational modification is important for optimal biological activity of the toxin.

D-Amino acids have a sporadic distribution in a number of biological systems. These have been characterized mostly in smaller peptides (for a comprehensive review on D-amino acids see Ref. 2), most notably those produced by frog skin, as well as by various invertebrate and mammalian sources. D-amino acids were described previously in the contryphans, a family of small peptides, which are widely distributed across the genus *Conus* (15–17). These small peptides (typically, eight amino acids) have a single disulfide bond; most have either a D-tryptophan or D-leucine residue (Table I). The contryphans were notable biochemically because of the slow equilibration between two conformational states exhibited by many of these peptides; the presence of a D-Trp residue was required for the slow conformational equilibrium.

The notable feature of the I-conotoxin superfamily, to which conotoxin r11a characterized above belongs, is the large number of different molecular isoforms differing in their primary sequences that can be present in a single venom. Conotoxin r11a is from the venom of *C. radiatus*, which is believed to be fish-hunting species. More than 20 distinct I-superfamily conotoxins, each significantly different in sequence, are expressed by *C. radiatus*. However, these share different extents of homology to the D-phenylalanine-containing conotoxin r11a characterized in this report. The modified phenylalanine residue is conserved in a subset of several I-superfamily conotoxins from *C. radiatus* and other *Conus* species, strongly suggesting that at least the members of this subset of I-superfamily conotoxins undergo the post-translational isomerization at this locus. The amino acids preceding the D-Phe residue are part of a conserved sequence motif, —GCSTSSSF— (where *F* is the D-Phe residue) (18).2

We have demonstrated that the D-Phe residue is important for the biological activity of conotoxin r11a. If an L-Phe, instead of a D-Phe is present, the peptide is significantly less active as an excitotoxin. A comparison of native and synthetic D-Phe-containing peptides was carried out. Since the effects of both

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2 M. Watkins and B. Olivera, unpublished results.
native and synthetic peptides are relatively irreversible, equilibrium dose-response measurements could not be obtained. Instead, as an index of toxin potency, the time interval between the administration of peptide and the first observation of repetitive activity (the induction time) was used. Higher concentrations of peptide had shorter induction times. At the same concentration, native and synthetic peptides had similar induction times. Furthermore, both peptides targeted channels in the nerve, rather than the muscle. Thus, both natural and synthetic peptides have comparable potencies and specificities. These functional features, along with their chemical characteristics, lead us to conclude that the natural and synthetic r11a are identical.

Although all of the I-superfamily peptides characterized so far appear to be excitotoxins, and several have been shown to target K channels in animals as diverse as mammals and stem chordates (22), it remains to be established which of the I-superfamily members (that all share the same Class 11 Cys pattern found in r11a) also contain d-amino acids. The discovery of a d-amino acid in conotoxin r11a introduces a cautionary note into using standard genetic/proteomic methods to investigate conotoxins: although the sequence of r11a and other I-superfamily conotoxins can be readily derived by PCR/cloning, and the number of hydroxylated Pro residues can be deduced by mass spectrometry, the presence of a d-amino acid would have eluded both of these methods.

We note that although the sequence of conotoxin r11a has no apparent homology to the ω-Aga-TK spider toxin, and the amino acids modified are different (Phe versus Ser), the locus of modification is the same distance from the C terminus (see Table I, A). In conotoxin r11a, which has 46 residues, it is a Phe residue at amino acid 44; in ω-Aga-TK, which has 48 residues, it is a Ser residue at amino acid 46. The characterization of a d-amino acid in conotoxin r11a raises the possibility that the third amino acid from the C terminus may be a favored site. This prediction is suggested by the discovery that another
I-superfamily conotoxin, with a Leu residue at the third position from the C terminus, has a D-Leu in the native peptide. It is noteworthy that in Table I, examples of peptides from different phyla are shown (Agelenopsis is a spider and Conus is a mollusc; Achatina and Mytilus are molluscs; Phyllomedusa is a frog; Ornithorhynchus is a platypus). This broad phylogenetic distribution, combined with the preferential loci of the amino acids modified, suggests that this post-translational isomerization, which occurs in many animal taxa, may be carried out by enzymatic systems that may be specific for a particular amino acid locus rather than the chemical nature of the amino acid isomerized.

A summary of the natural occurrence of D-amino acids in polypeptides is shown in Table I. The evidence that D-amino acids found in most polypeptides are formed post-translationally has been reviewed (2). In many cases, mRNA encoding a precursor for the peptide containing the D-amino acid was identified. (The first peptide where post-translational modification was definitely established was dermorphin in 1987 by Gunther Kreil’s laboratory (23).)

Enzymatic activities have been purified both from spider venoms (24) and from frog skin secretions that specifically convert one L-amino acid to the D-amino acid in the

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**Table I**

**Peptides containing D-amino acids**

| Peptide                | Sequence                                                                 | Source                          | Ref. |
|------------------------|---------------------------------------------------------------------------|---------------------------------|------|
| A. Peptides with a D-amino acid at position 3 from the C terminus |                                                                          |                                 |      |
| α-Agatoxin TK           | EDNCIAEDYGKCTWGGTKCCRGRPSCSMMTNCECTPRLIMEGLSFA†                           | Agelenopsis aperta (3)          |      |
| r11a                   | GOSFCKADEKOCEYHADCCNCLSLGICAOSTNWILPGCSTSSFFK†                            | C. radiatus                     |      |
| Achatin-I              | GFAD†‡‡                                   | Achatina fulica (8)             |      |
|                        |                                                                          |                                 |      |
| B. Peptides with a D-amino acid at position 2 from the N terminus |                                                                          |                                 |      |
| Fulcin                 | FNEF†‡                                   | Achatina fulica (11)            |      |
| Achatin-I              | GFAD†‡                                   | Achatina fulica (8)             |      |
| Mytilus-FFRF amide     | ALAGDHHFFRF†‡                            | Mytilus edulis (12)             |      |
| Dermorphin             | YAGYPS†‡                                | Phyllomedusa sauvagei (7)       |      |
| Met-deltorphin         | YMFHLMD†‡                               | Phyllomedusa sauvagei (9, 10)   |      |
| Platypus peptide       | HEDHNFKNKKPMKGLSKGCFLKLDRIGOSTSGLC†                                            | Ornithorhynchus anatinus (15, 14) |      |
| C. Peptides with a D-amino acid at position 4 from the N terminus |                                                                          |                                 |      |
| Contraryphan-R         | GCQEPWPC†‡                               | C. radiatus                     |      |
| Contraryphan-Sm        | GCQWPWPC†‡                               | Conus stercusmuscureum (16)    |      |
| Leu-Contraryphan-P     | GCVYLPWPC†‡                             | Conus purpurascens (17)         |      |

† The underlined letter indicates D-isomers.
‡ O, 4-trans-hydroxyproline.
‡‡ C-terminal free acid.
‡‡‡ C-terminal amidation.

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3 O. Buczek and E. Jimenez, manuscript in preparation.
4 A. Jilek and G. Kreil, personal communication.
peptide precursor. The d-amino acids found in conotoxins are therefore presumed to be produced post-translationally. In most cases, the relevant cDNA clones encoding these conotoxins with d-amino acids have been identified.

It is noteworthy that C. radiatus, the organism used for the present study, is the first in which two post-translationally modified polypeptides have been characterized that differ in their d-amino acids (Phe in the present peptide, Trp in contrysphan-R), as well as in the loci in which these d-amino acids are found on the polypeptide chain. This raises the possibility that different enzymatic activities catalyze the isomerization from L- to D-amino acids in the two C. radiatus peptides; whether these are related to the factors identified in the spider and frog systems remains to be established.

REFERENCES
1. Kreil, G. (1994) J. Biol. Chem. 269, 10967–10970
2. Kreil, G. (1997) Annu. Rev. Biochem. 66, 337–345
3. Kuwada, M., Teramoto, T., Kumagaye, K. Y., Nakajima, K., Watanabe, T., Kawai, T., Kawakami, Y., Nödeme, T., Sawada, K., Nishizawa, Y., and Katayama, K. (1994) Mol. Pharmacol. 46, 587–593
4. Soyez, D., Van Herp, F., Rossier, J., Le Caer, J.-P., Tensen, C. P., and Lafont, R. (1994) J. Biol. Chem. 28, 18295–18298
5. Yasuda, A., Yasuda, Y., Fujita, T., and Naya, Y. (1994) Gen. Comp. Endocrinol. 95, 387–398
6. Aguilar, M. B., Soyez, D., Falchetto, R., Arnott, D., Shabanowitz, J., Hunt, D. F., and Huberman, A. (1995) Peptides 16, 1375–1383
7. Montecuccci, P. C., de Castiglione, R., Piani, S., Gozzini, L., and Erspamer, V. (1981) Int. J. Pept. Prot. Res. 17, 275–283
8. Kamatani, Y., Minakata, H., Kenny, P. T. M., Iwasita, T., Watanabe, K., Funase, K., Sun, Y. P., Yongzui, A., Kim, K. H., Nohales-Li, P., Nohales, E. T., Kanapi, C. G., Takeuchi, H., and Nomoto, K. (1989) Biochem. Biophys. Res. Commun. 160, 1015–1020
9. Kreil, G., Barra, D., Simmaco, M., Espamer, V., Falconieri-Erspamer, G., Negri, L., Severini, C., Corsi, R., and Melchiorri, P. (1989) Eur. J. Pharmacol. 162, 123–128
10. Mor, A., Deltour, A., Sagan, S., Amiche, M., Pradelles, P., Rossier, J., and Nicholas, P. (1989) FEBS Lett. 255, 269–274
11. Ohta, N., Kubota, I., Takao, T., Shimoniishi, Y., Yasuda-Kamatani, Y., Minakata, H., Nomoto, K., Muneoka, Y., and Kobayashi, M. (1991) Biochem. Biophys. Res. Commun. 178, 486–493
12. Fujisawa, I., Ikeda, T., Nomoto, K., Yasuda-Kamatani, Y., Minakata, H., Kenny, P. T. M., Kubota, I., and Muneoka, Y. (1992) Comp. Biochem. Physiol. (C) 102, 91–95
13. de Plater, G. M., Martin, R. L., and Milburn, P. J. (1998) Comp. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 120, 99–110
14. Torres, A. M., Menz, I., Averwood, P. F., Balsal, P., Lahmstein, J., Gallagher, C. H., and Kuchel, P. W. (2002) FEBS Lett. 524, 172–176
15. Jimenez, E. C., Olivera, B. M., Gray, W. R., and Cruz, L. J. (1996) J. Biol. Chem. 281, 28002–28005
16. Jacobsen, R., Jimenez, E. C., Grilley, M., Watkins, M., Hillyard, D., Cruz, L. J., and Olivera, B. M. (1998) J. Peptide Res. 51, 173–179
17. Jacobsen, R., Jimenez, E. C., DeaCruz, R. G. C., Gray, W. R., Cruz, L. J., and Olivera, B. M. (1999) J. Peptide Res. 54, 93–99
18. Jimenez, E. C., Shetty, R. P., Lirazan, M., Rivier, J., Walker, C., Abogadie, F. C., Yoshikami, D., Cruz, L. J., and Olivera, B. M. (2003) J. Neurochem. 85, 610–621
19. Atherton, E., and Sheppard, R. C. (1989) Solid-Phase Peptide Synthesis, A Practical Approach, IRL Press, Oxford
20. Clark, C., Olivera, B. M., and Cruz, L. J. (1981) Toxicon 19, 691–699
21. Yoshikami, D., Bagabaldo, Z., and Olivera, B. M. (1989) Ann. N. Y. Acad. Sci. 560, 230–248
22. Kauferstein, S., Huys, I., Lanthanh, H., Stöcklin, R., Sotto, F., Menez, A., Tytgat, J., and Mebs, D. (2003) Toxicon 42, 43–52
23. Richter, K., Egger, R., and Kreil, G. (1987) Science 238, 200–202
24. Heck, S. D., Kelbaugh, P. R., Kelly, M. E., Thadeio, P. F., Saccamano, N. A., Stroh, J. G., and Volkman, R. A. (1994) J. Am. Chem. Soc. 116, 10348–10356
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