Letters

A Novel N-Terminal Cyclic Dynorphin A Analogue *cyclo*^{N,5}[Trp^3,Trp^4,Glu^5] Dynorphin A-(1−11)NH$_2$ That Lacks the Basic N-Terminus

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Abstract: A novel N-terminal-to-side chain cyclic dynorphin A analogue lacking the basic N-terminus was designed based on Ac[Lys^2,Trp^3,Trp^4,D-Ala^8]dynorphin A-(1−11)NH$_2$ (Wan et al. *J. Med. Chem.* **1999**, *42*, 3011−3013). *cyclo*^{N,5}-[Trp^3,Trp^4,Glu^5]dynorphin A-(1−11)NH$_2$ showed similar κ opioid receptor affinity ($K_i = 27$ nM) and selectivity (K_i ratio ($\kappa/\mu/\delta$) = 1/12/330) to the linear peptide and antagonized dynorphin A-(1−13)NH$_2$ at κ opioid receptors. This is the first opioid peptide cyclized through the N-terminus that retains high opioid receptor affinity.

Opioid receptors (κ, μ, and δ), which are distributed throughout the central and peripheral nervous systems, are involved in a variety of physiological processes.[1] One of the most important functions of these receptors is in analgesia. Mu opioid receptor agonists such as morphine are used extensively for the treatment of severe pain, but serious side effects, namely respiratory depression, constipation, and physical dependence, are associated with these agents. Therefore, ligands for other opioid receptors have been explored in attempts to overcome the limitations of current analgesics and for other therapeutic applications.[2]

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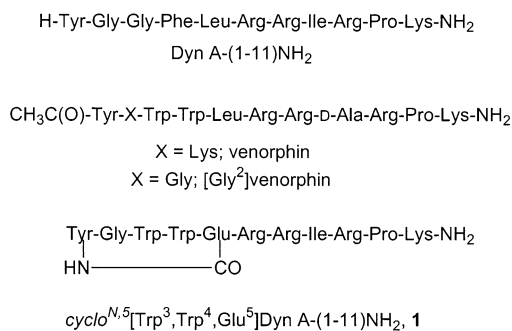 § University of Georgia.
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Kappa opioid receptor ligands, both agonists and antagonists, have been examined as both pharmacological tools and as potential therapeutic agents.[2] Kappa opioid receptor agonists produce analgesia without the clinical side effects observed with μ opioid agonists,[3] but the clinical utility of centrally acting κ opioid receptor analgesics has often been limited by psychotomimetic effects.[4] Recently there has been considerable interest in developing peripherally selective κ opioid receptor agonists as analgesics.[2,5−7] Agonists for κ opioid receptors may also have other therapeutic applications,[8−10] including the treatment of cocaine dependence.[11−13] Kappa opioid receptor antagonists are useful pharmacological tools for studying the functions of κ receptors at the molecular level, which in turn could be very important in the development of new therapeutic agents. Recently, evidence has also been presented that κ opioid antagonists may be useful in the treatment of opioid addiction.[14]

While a variety of κ opioid receptor selective agonists have been described,[2] reports of κ opioid receptor selective antagonists have been very limited. Most studies have used the nonpeptide κ opioid receptor antagonist nor-binaltorphimine (nor-BNI),[15,16] but the selectivity of this ligand for κ opioid receptors in vitro is only modest (K_e ratio for μ/κ and δ/κ in smooth muscle assays = 20−35)[17,18] and in vivo it exhibits a relatively low potency and unusual pharmacokinetic properties (a slow onset, time dependent selectivity, and a very long duration of action) that complicate its use as a pharmacological tool (see ref 19). Recently C5′-guanidino-naltrindole (GNTI)[17,18,20] was reported to have greater selectivity for κ opioid receptors and increased potency in vivo than norBNI,[19] but in vivo GNTI also has a slow onset and long half-life with peak activity not observed until after 24 h.[19]

We are interested in developing conformationally constrained peptides with antagonist activity at κ opioid receptors. Such constrained peptides are very useful in identifying possible bioactive conformations and in developing pharmacophoric models for receptor−ligand interactions. Based on the novel acetylated chimeric dynorphin A (Dyn A) analogue Ac[Lys^2,Trp^3,Trp^4,D-Ala^8]-dynorphin A-(1−11)NH$_2$ (JVA-901, now called venor-

H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-NH$_2$

Dyn A-(1-11)NH$_2$

CH$_3$C(O)-Tyr-X-Trp-Trp-Leu-Arg-Arg-D-Ala-Arg-Pro-Lys-NH$_2$

X = Lys; venorphin

X = Gly; [Gly^2]venorphin

Tyr-Gly-Trp-Trp-Glu-Arg-Arg-Ile-Arg-Pro-Lys-NH$_2$

HN————————CO

cyclo^{N,5}[Trp^3,Trp^4,Glu^5]Dyn A-(1-11)NH$_2$, **1**

Figure 1. Sequence similarities between Dyn A-(1−11)NH$_2$, venorphin, [Gly^2]venorphin, and the head-to-side chain cyclic Dyn A analogue *cyclo^{N,5}*[Trp^3,Trp^4,Glu^5]Dyn A-(1−11)NH$_2$, **1**.

phin, Figure 1) which exhibits antagonist activity,[21] we designed a constrained derivative cyclized through the N-terminus as an antagonist at κ opioid receptors. Here we describe the synthesis and pharmacological profile of this novel N-terminal cyclic Dyn A analogue.

Although a variety of peptide agonists for κ opioid receptors have been identified, including some with high κ opioid receptor selectivity,[22−25] the search for peptide antagonists for these receptors met with limited success until very recently. Early analogues exhibited weak antagonist activity, residual agonist activity, and/or low selectivity for κ opioid receptors.[24,26−29] Using the "message-address" concept,[30,31] the acetylated chimeric Dyn A analogue venorphin (Figure 1) was designed and synthesized in our laboratory.[21] This peptide shows nanomolar affinity and selectivity for κ opioid receptors in binding assays and exhibits reduced efficacy and antagonist activity in the adenylyl cyclase assay using cloned κ opioid receptors. Interestingly this peptide lacks the basic N-terminal amine which appears to be critical

for the opioid agonist activity of Dyn A.[27] More recently, Schiller and co-workers reported a des-amino analogue of Dyn A-(1−11)NH$_2$, dynantin, that shows high selectivity and potent antagonist activity at κ opioid receptors.[32] Substitution of Pro at position 3 of Dyn A-(1−11)NH$_2$ also results in a peptide which is very selective for κ opioid receptors, but this analogue exhibits weak antagonist activity.[25] Selective peptide antagonists are complimentary to nonpeptide antagonists as pharmacological tools to study κ opioid receptors, and studying these peptides may reveal distinct receptor−ligand interactions that can be utilized in future ligand design. The conformational flexibility of these linear peptides, however, complicates the evaluation of possible spatial relationships of pharmacophoric groups in the peptides.

Like most linear peptides, Dyn A is capable of assuming a number of different conformations,[33−38] and the biologically active conformations of Dyn A are not yet clear. This inherent conformational flexibility of Dyn A, which permits the peptide to adopt different conformations in different environments (i.e., in different opioid receptor binding sites), may be one of the reasons for the low κ opioid receptor selectivity of Dyn A.[39,40] To restrict the flexibility of Dyn A, various side chain-to-side chain cyclic analogues of Dyn A have been synthesized,[41−46] but none of these peptides have constrained the critical Tyr^1 residue. Also to date, none of the reported cyclic analogues of Dyn A have shown antagonist activity. Cyclic Dyn A analogues with antagonist activity will be useful for comparison to cyclic peptide agonists and the evaluation of possible differences in the bioactive conformations of opioid peptides with different efficacies at κ opioid receptors.
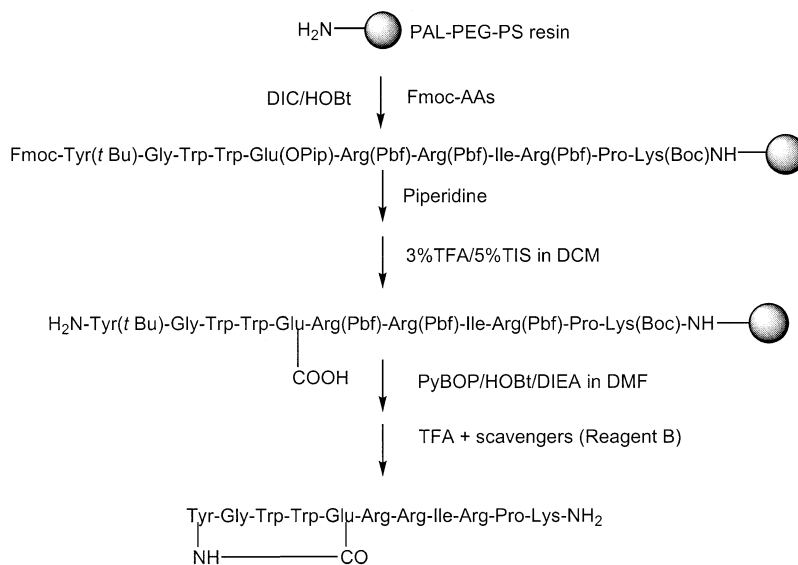
Since a basic N-terminal amine is not required for κ opioid receptor affinity or antagonist activity,[21,32] our

Table 1. Opioid Receptor Binding Affinities^a

Dyn A(1-11) NH$_2$ analogues	K_i (nM ± SEM)			K_i ratio ($\kappa/\mu/\delta$)
	κ	μ	δ	
cyclo^{N,5}[Trp^3,Trp^4,Glu^5], **1**	26.8 ± 2.8	331 ± 29	>8900	1/12/>330
[Gly^2]venorphin	8.4 ± 0.8	339 ± 60	>10000	1/40/>1190
venorphin	19.8 ± 5.2	251 ± 22	5320 ± 1130	1/13/270

^{a} Radioligands: [^{3}H]Diprenorphine, [^{3}H]DAMGO ([D-Ala^2,MePhe^4,glyol]enkephalin), and [^{3}H]DPDPE (*cyclo*[D-Pen^2,D-Pen^5]enkephalin) were used as radioligands in assays for κ, μ, and δ receptors, respectively. Results are average ± SEM of n = 3 independent experiments.

Scheme 1. Synthesis of *cyclo^{N,5}*[Trp^3,Trp^4,Glu^5]Dyn A-(1−11)NH$_2$, **1**

H$_2$N——◯ PAL-PEG-PS resin

DIC/HOBt | Fmoc-AAs

Fmoc-Tyr(*t* Bu)-Gly-Trp-Trp-Glu(OPip)-Arg(Pbf)-Arg(Pbf)-Ile-Arg(Pbf)-Pro-Lys(Boc)NH——◯

| Piperidine

| 3%TFA/5%TIS in DCM

H$_2$N-Tyr(*t* Bu)-Gly-Trp-Trp-Glu-Arg(Pbf)-Arg(Pbf)-Ile-Arg(Pbf)-Pro-Lys(Boc)-NH——◯

COOH | PyBOP/HOBt/DIEA in DMF

| TFA + scavengers (Reagent B)

Tyr-Gly-Trp-Trp-Glu-Arg-Arg-Ile-Arg-Pro-Lys-NH$_2$

NH————————CO

hypothesis was that cyclization through a nonbasic N-terminus could be used to restrict the conformation of Tyr^1 while still retaining κ opioid receptor affinity; based on the activity of venorphin, the resulting cyclic peptide was expected to be an antagonist. Therefore, the novel N-terminal-to-side chain cyclic analogue $cyclo^{N,5}$-$[Trp^3,Trp^4,Glu^5]Dyn$ A-(1−11)NH_2 (**1**, Figure 1) with a nonbasic N-terminus was chosen for synthesis. Since Leu^5 is a noncritical residue in Dyn A,[47] the cyclization was performed between the N-terminal amine and the side chain of a glutamic acid residue incorporated at position 5. The initial structure−activity relationship (SAR) study of venorphin indicated that Lys at position 2 was not important for opioid receptor affinity,[48] and in fact $Ac[Trp^3,Trp^4,D\text{-}Ala^8]Dyn$ A-(1−11)NH_2 ($[Gly^2]$-venorphin) has higher affinity (K_i = 8.4 nM) and selectivity for κ opioid receptors than venorphin (Table 1).[48] Therefore the N-terminal-to-side chain cyclic analogue based on this venorphin derivative was synthesized and evaluated for opioid receptor affinity and efficacy.

The cyclic peptide was synthesized by solid-phase synthesis on a PAL−PEG−PS (peptide amide linker−poly(ethylene glycol)−polystyrene) resin using Fmoc (9-fluorenylmethoxycarbonyl)-protected amino acids. The side chain of Glu was protected as the Pip (phenyliso-propyl) ester, and the peptide was synthesized as shown in Scheme 1. Once the protected full-length linear peptide was assembled, the N-terminal Fmoc group was removed, followed by selective deprotection of the side chain of Glu with dilute TFA (trifluoroacetic acid) (3% TFA/5% TIS (triisopropylsilane) in DCM (dichloro-methane, 2 × 1 h).[49] The cyclic peptide was obtained by cyclization on the resin using a 10-fold excess of PyBOP (benzotriazol-1-yl-oxy-tris-pyrrolidino-phospho-nium hexafluorophosphate)/HOBt (1-hydroxybenzotria-zole)/DIEA (*N,N*-diisopropylethylamine) (1:1:2, 0.2 M) in DMF (*N,N*-dimethylformamide) for 10 h. The peptide was cleaved from the resin using Reagent B (88% TFA, 5% phenol, 5% water, and 2% TIS)[50] and purified by preparative reversed phase HPLC.[51]

The peptides were examined for their opioid receptor affinity as described previously[46] using Chinese hamster ovary (CHO) cells stably expressing cloned opioid receptors (Table 1). $cyclo^{N,5}[Trp^3,Trp^4,Glu^5]Dyn$ A-(1−11)NH_2, **1**, showed similar opioid receptor affinity and selectivity as venorphin and only a 3-fold lower affinity for κ receptors compared to its linear counterpart $[Gly^2]$-venorphin. This is the first head-to-side chain cyclic analogue of an opioid peptide that lacks the basic N-terminal amine and still shows nanomolar affinity for opioid receptors. Like venorphin, the cyclic peptide showed very low affinity for δ opioid receptors, resulting in high selectivity for κ over δ opioid receptors. The affinity of the cyclic peptide for μ receptors was similar to that of venorphin, so that the cyclic peptide and venorphin exhibited similar selectivities for κ over μ opioid receptors.

The cyclic peptide was evaluated for efficacy in an adenylyl cyclase assay as previously described[52] using cloned rat κ opioid receptors stably expressed in CHO cells. Compound **1** exhibited negligible efficacy in this assay (maximum inhibition 8 ± 8% (n = 2) relative to Dyn A(1−13)NH_2), considerably less than that exhibited by the linear peptide venorphin (28 ± 13% maximum inhibition)[21] and also less than $[Gly^2]$venorphin (15 ± 5% maximum inhibition). Therefore the cyclic peptide
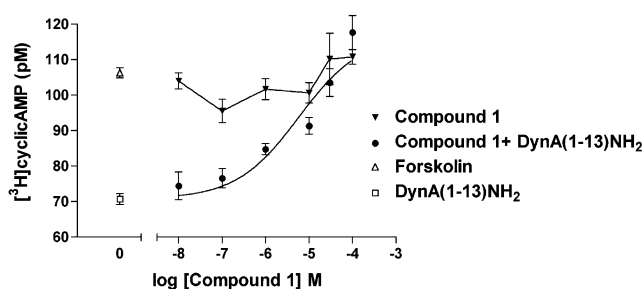

Figure 2. Inhibition of cAMP production by $cyclo^{N,5}$-$[Trp^3,Trp^4,Glu^5]Dyn$ A-(1−11)NH_2, **1** (▼) and Dyn A (1−13)-NH_2 (□) in CHO cells expressing κ opioid receptors and reversal by $cyclo^{N,5}[Trp^3,Trp^4,Glu^5]Dyn$ A-(1−11)NH_2 (●) of the inhibition of cAMP production by 10 nM Dyn A (1−13)NH_2. Data are the mean ± SEM of triplicate determinations from a representative assay.

was evaluated for antagonist activity in this assay; it completely reversed the agonist activity of 10 nM Dyn A (1−13)NH_2 in a concentration-dependent manner (Figure 2).

In conclusion, the first head-to-side chain cyclic analogue of an opioid peptide that retains opioid receptor affinity was prepared. $cyclo^{N,5}[Trp^3,Trp^4,Glu^5]Dyn$ A-(1−11)NH_2, **1**, which lacks the basic N-terminal amine exhibits nanomolar κ opioid receptor affinity, selectivity comparable to venorphin, and antagonist activity in the adenylyl cyclase assay. $cyclo^{N,5}[Trp^3,$-$Trp^4,Glu^5]Dyn$ A-(1−11)NH_2 is the first cyclic peptide antagonist for κ opioid receptors and is a promising lead peptide which can be further modified and studied for its interactions with κ opioid receptors. These studies are currently ongoing in our laboratory.

References

(1) Dhawan, B. N.; Cesselin, F.; Raghubir, R.; Reisine, T.; Bradley, P. B.; Portoghese, P. S.; Hamon, M. International Union of Pharmacology. XII. Classification of Opioid Receptors. *Pharmacol. Rev.* **1996**, *48*, 567−592.

(2) Aldrich, J. V.; Vigil-Cruz, S. C. Narcotic Analgesics. *Burger's Medicinal Chemistry and Drug Discovery*; Abraham, D., Ed.; John Wiley & Sons: New York, 2003; in press.

(3) Millan, M. J. Kappa-Opioid Receptors and Analgesia. *Trends Pharmacol. Sci.* **1990**, *11*, 70−76.

(4) Pfeiffer, A.; Brantl, V.; Herz, A.; Emrich, H. M. Psychotomimesis Mediated by Kappa Opiate Receptors. *Science* **1986**, *233*, 774−776.

(5) Machelska, H.; Pfluger, M.; Weber, W.; Piranvisseh-Volk, M.; Daubert, J. D.; Dehaven, R.; Stein, C. Peripheral Effects of the Kappa-Opioid Agonist EMD 61753 on Pain and Inflammation in Rats and Humans. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 354−361.

(6) Ko, M. C.; Butelman, E. R.; Woods, J. H. Activation of Peripheral Kappa Opioid Receptors Inhibits Capsaicin-Induced Thermal Nociception in Rhesus Monkeys. *J. Pharmacol. Exp. Ther.* **1999**, *289*, 378−385.

(7) Ko, M. C.; Willmont, K. J.; Burritt, A.; Hruby, V. J.; Woods, J. H. Local Inhibitory Effects of Dynorphin A-(1−17) on Capsaicin-Induced Thermal Allodynia in Rhesus Monkeys. *Eur. J. Pharmacol.* **2000**, *402*, 69−76.

(8) Chao, C. C.; Gekker, G.; Hu, S.; Sheng, W. S.; Shark, K. B.; Bu, D.-F.; Archer, S.; Bidlack, J. M.; Peterson, P. K. Kappa Opioid Receptors in Human Microglia Downregulate Human Immunodeficiency Virus 1 Expression. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, *93*, 8051−8056.

(9) Peterson, P. K.; Gekker, G.; Lokensgard, J. R.; Bidlack, J. M.; Chang, A. C.; Fang, X.; Portoghese, P. S. Kappa-Opioid Receptor Agonist Suppression of HIV-1 Expression in CD4+ Lymphocytes. *Biochem. Pharmacol.* **2001**, *61*, 1145−1151.

(10) Tortella, F. C.; Decoster, M. A. Kappa Opioids: Therapeutic Considerations in Epilepsy and CNS Injury. *Clin. Neuropharmacol.* **1994**, *17*, 403–416.

(11) Negus, S. S.; Mello, N. K.; Portoghese, P. S.; Lin, C. E. Effects of Kappa Opioids on Cocaine Self-Administration by Rhesus Monkeys. *J. Pharmacol. Exp. Ther.* **1997**, *282*, 44–55.

(12) Schenk, S.; Partridge, B.; Shippenberg, T. S. U69593, a Kappa-Opioid Agonist, Decreases Cocaine Self-Administration and Decreases Cocaine-Produced Drug-Seeking. *Psychopharmacol. (Berl.)* **1999**, *144*, 339–346.

(13) Mello, N. K.; Negus, S. S. Interactions between Kappa Opioid Agonists and Cocaine. Preclinical Studies. *Ann. N. Y. Acad. Sci* **2000**, *909*, 104–132.

(14) Rothman, R. B.; Gorelick, D. A.; Heishman, S. J.; Eichmiller, P. R.; Hill, B. H.; Norbeck, J.; Liberto, J. G. An Open-Label Study of a Functional Opioid Kappa Antagonist in the Treatment of Opioid Dependence. *J. Subst. Abuse Treat.* **2000**, *18*, 277–281.

(15) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. Binaltorphimine and Nor-Binaltorphimine, Potent and Selective Kappa-Opioid Receptor Antagonists. *Life Sci.* **1987**, *40*, 1287–1292.

(16) Portoghese, P. S.; Lipkowski, A. W.; Takemori, A. E. Bimorphinans as Highly Selective, Potent Kappa Opioid Receptor Antagonists. *J. Med. Chem.* **1987**, *30*, 238–239.

(17) Jones, R. M.; Hjorth, S. A.; Schwartz, T. W.; Portoghese, P. S. Mutational Evidence for a Common Kappa Antagonist Binding Pocket in the Wild-Type Kappa and Mutant Mu[K303E] Opioid Receptors. *J. Med. Chem.* **1998**, *41*, 4911–4914.

(18) Stevens, W. C., Jr.; Jones, R. M.; Subramanian, G.; Metzger, T. G.; Ferguson, D. M.; Portoghese, P. S. Potent and Selective Indolomorphinan Antagonists of the Kappa-Opioid Receptor. *J. Med. Chem.* **2000**, *43*, 2759–2769.

(19) Negus, S. S.; Mello, N. K.; Linsenmayer, D. C.; Jones, R. M.; Portoghese, P. S. Kappa Opioid Antagonist Effects of the Novel Kappa Antagonist 5′-Guanidinonaltrindole (GNTI) in an Assay of Schedule-Controlled Behavior in Rhesus Monkeys. *Psychopharmacol. (Berl.)* **2002**, *163*, 412–419.

(20) Jones, R. M.; Portoghese, P. S. 5′-Guanidinonaltrindole, a Highly Selective and Potent Kappa-Opioid Receptor Antagonist. *Eur. J. Pharmacol.* **2000**, *396*, 49–52.

(21) Wan, Q.; Murray, T. F.; Aldrich, J. V. A Novel Acetylated Analogue of Dynorphin A-(1–11) Amide as a Kappa-Opioid Receptor Antagonist. *J. Med. Chem.* **1999**, *42*, 3011–3013.

(22) Choi, H.; Murray, T. F.; DeLander, G. E.; Caldwell, V.; Aldrich, J. V. N-Terminal Alkylated Derivatives of [D-Pro10]Dynorphin A-(1–11) are Highly Selective for Kappa-Opioid Receptors. *J. Med. Chem.* **1992**, *35*, 4638–4639.

(23) Lung, F.-D. T.; Meyer, J.-P.; Li, G.; Lou, B.-S.; Stropova, D.; Davis, P.; Yamamura, H. I.; Porreca, F.; Hruby, V. J. Highly Kappa Receptor-Selective Dynorphin A Analogues with Modifications in Position 3 of Dynorphin A(1–11)-NH2. *J. Med. Chem.* **1995**, *38*, 585–586.

(24) Choi, H.; Murray, T. F.; DeLander, G. E.; Schmidt, W. K.; Aldrich, J. V. Synthesis and Opioid Activity of [D-Pro10]-Dynorphin A-(1–11) Analogues with N-Terminal Alkyl Substitution. *J. Med. Chem.* **1997**, *40*, 2733–2739.

(25) Schlechtingen, G.; Zhang, L.; Maycock, A.; DeHaven, R. N.; Daubert, J. D.; Cassel, J.; Chung, N. N.; Schiller, P. W.; Goodman, M. [Pro3]Dynorphin A(1–11)NH2: A Dynorphin Analogue with High Selectivity for the Kappa Opioid Receptor. *J. Med. Chem.* **2000**, *43*, 2698–2702.

(26) Gairin, J. E.; Mazarguil, H.; Alvinerie, P.; Botanch, C.; Cros, J.; Meunier, J. N,N-Diallyl-Tyrosyl Substitution Confers Antagonist Properties on the Kappa-Selective Opioid Peptide [D-Pro10]-dynorphin A-(1–11). *Br. J. Pharmacol.* **1988**, *95*, 1023–1030.

(27) Kawasaki, A. M.; Knapp, R. J.; Walton, A.; Wire, W. S.; Zalewska, T.; Yamamura, H. I.; Porreca, F.; Burks, T. F.; Hruby, V. J. Synthesis, Opioid Binding Affinities, and Potencies of Dynorphin A Analogues Substituted in Positions 1, 6, 7, 8 and 10. *Int. J. Pept. Protein Res.* **1993**, *42*, 411–419.

(28) Lemaire, S.; Turcotte, A. Synthesis and Biological Activity of Analogues of Dynorphin-A(1–13) Substituted in Positions 2 and 4: Design of [Ala2,Trp4]-Dyn-A(1–13) as a Putative Selective Opioid Antagonist. *Can. J. Physiol. Pharmacol.* **1986**, *64*, 673–678.

(29) Gairin, J. E.; Mazarguil, H.; Alvinerie, P.; St.-Pierre, S.; Meunier, J.-C.; Cros, J. Synthesis and Biological Activity of Dynorphin A Analogues with Opioid Antagonist Properties. *J. Med. Chem.* **1986**, *29*, 1913–1917.

(30) Chavkin, C.; Goldstein, A. Specific Receptor for the Opioid Peptide Dynorphin: Structure–Activity Relationships. *Proc. Natl. Acad. Sci. U. S. A.* **1981**, *78*, 6543–6547.

(31) Schwyzer, R. Acth: A Short Introductory Review. *Ann. N. Y. Acad. Sci.* **1977**, *297*, 3–26.

(32) Lu, Y.; Nguyen, T. M.-D.; Weltrowska, G.; Berezowska, I.; Lemieux, C.; Chung, N. N.; Schiller, P. W. [2′,6′-Dimethyl-tyrosine]Dynorphin A(1–11)-NH2 Analogues Lacking an N-Terminal Amino Group: Potent and Selective Kappa Opioid Antagonists. *J. Med. Chem.* **2001**, *44*, 3048–3053.

(33) Maroun, R.; Mattice, W. L. Solution Conformations of the Pituitary Opioid Peptide Dynorphin-(1–13). *Biochem. Biophys. Res. Commun.* **1981**, *103*, 442–446.

(34) Zhou, N.; Gibbons, W. A. A 1H Nuclear Magnetic Resonance Study of the Opioid Peptide Dynorphin-(1–13) in Aqueous Solution. *J. Chem. Soc., Perkin Trans. 2* **1986**, 637–644.

(35) Schwyzer, R. Estimated Conformation, Orientation, and Accumulation of Dynorphin A-(1–13)-Tridecapeptide on the Surface of Neutral Lipid Membranes. *Biochemistry* **1986**, *25*, 4281–4286.

(36) Kallick, D. A. Conformation of Dynorphin A(1–17) Bound to Dodecylphosphocholine Micelles. *J. Am. Chem. Soc.* **1993**, *115*, 9317–9318.

(37) Tessmer, M. R.; Kallick, D. A. NMR and Structural Model of Dynorphin A(1–17) Bound to Dodecylphosphocholine Micelles. *Biochemistry* **1997**, *36*, 1971–1981.

(38) Spadaccini, R.; Crescenzi, O.; Picone, D.; Tancredi, T.; Temussi, P. A. Solution Structure of Dynorphin A (1–17): A NMR Study in a Cryoprotective Solvent Mixture at 278 K. *J. Pept. Sci.* **1999**, *5*, 306–312.

(39) Hruby, V. J.; Al-Obeidi, F.; Kazmierski, W. Emerging Approaches in the Molecular Design of Receptor-Selective Peptide Ligands: Conformational, Topographical and Dynamic Considerations. *Biochem. J.* **1990**, *268*, 249–262.

(40) Schiller, P. W. Development of Receptor-Specific Opioid Peptide Analogues. *Prog. Med. Chem.* **1991**, *28*, 301–340.

(41) Kawasaki, A. M.; Knapp, R. J.; Kramer, T. H.; Wire, W. S.; Vasquez, O. S.; Yamamura, H. I.; Burks, T. F.; Hruby, V. J. Design and Synthesis of Highly Potent and Selective Cyclic Dynorphin A Analogues. *J. Med. Chem.* **1990**, *33*, 1874–1879.

(42) Kawasaki, A. M.; Knapp, R. J.; Kramer, T. H.; Walton, A.; Wire, W. S.; Hashimoto, S.; Yamamura, H. I.; Porreca, F.; Burks, T. F.; Hruby, V. J. Design and Synthesis of Highly Potent and Selective Cyclic Dynorphin A Analogues. 2. New Analogues. *J. Med. Chem.* **1993**, *36*, 750–757.

(43) Meyer, J. P.; Collins, N.; Lung, F. D.; Davis, P.; Zalewska, T.; Porreca, F.; Yamamura, H. I.; Hruby, V. J. Design, Synthesis, and Biological Properties of Highly Potent Cyclic Dynorphin A Analogues. Analogues Cyclized between Positions 5 and 11. *J. Med. Chem.* **1994**, *37*, 3910–3917.

(44) Arttamangkul, S.; Murray, T. F.; DeLander, G. E.; Aldrich, J. V. Synthesis and Opioid Activity of Conformationally Constrained Dynorphin A Analogues. 1. Conformational Constraint in the "Message" Sequence. *J. Med. Chem.* **1995**, *38*, 2410–2417.

(45) Lung, F.-D. T.; Collins, N.; Stropova, D.; Davis, P.; Yamamura, H. I.; Porreca, F.; Hruby, V. J. Design, Synthesis, and Biological Activities of Cyclic Lactam Peptide Analogues of Dynorphin A(1–11)-NH2. *J. Med. Chem.* **1996**, *39*, 1136–1141.

(46) Arttamangkul, S.; Ishmael, J. E.; Murray, T. F.; Grandy, D. K.; DeLander, G. E.; Kieffer, B. L.; Aldrich, J. V. Synthesis and Opioid Activity of Conformationally Constrained Dynorphin A Analogues. 2. Conformational Constraint in the "Address" Sequence. *J. Med. Chem.* **1997**, *40*, 1211–1218.

(47) Turcotte, A.; Lalonde, J.-M.; St-Pierre, S.; Lemaire, S. Dynorphin-(1–13). I. Structure–Function Relationships of Ala-Containing Analogues. *Int. J. Pept. Protein Res.* **1984**, *23*, 361–367.

(48) Aldrich, J. V.; Wan, Q.; Murray, T. F. Novel Opioid Peptides as Kappa Opioid Receptor Antagonists. *Peptides: Chemistry and Biology*; Barany, G., Fields, G., Eds.; ESCOM: Leiden, 2000; pp 616–618.

(49) Yue, C.; Thierry, J.; Potier, P. 2-Phenyl Isopropyl Esters as Carboxyl Terminus Protecting Groups in the Fast Synthesis of Peptide Fragments. *Tetrahedron Lett.* **1993**, *34*, 323–326.

(50) Solé, N. A.; Barany, G. Optimization of Solid-Phase Synthesis of [Ala8]-Dynorphin A. *J. Org. Chem.* **1992**, *57*, 5399–5403.

(51) Analytical data for $cyclo^{N,5}$[Trp3,Trp4,Glu5]Dyn A-(1–11)NH2: ESI-MS m/z (M + H)+ 1528.7 (1528.7 calcd); HPLC system 1 t_r = 20.3 min (5–80% solvent B over 50 min) purity = 97%; HPLC system 2 t_r = 31.2 min (5–80% solvent B over 50 min) purity = 97%. ESI-MS spectra were obtained on a Thermoquest LCQ electrospray ionization mass spectrometer (ESI-MS). HPLC system 1, solvent A = 0.1% aqueous TFA and solvent B = 0.1% TFA in MeCN (flow rate = 1 mL/min); HPLC system 2, solvent A = 0.1% aqueous TFA and solvent B = 0.1% TFA in MeOH (flow rate = 1 mL/min).

(52) Soderstrom, K.; Choi, H.; Aldrich, J. V.; Murray, T. F. N-Alkylated Derivatives of [D-Pro10]Dynorphin A-(1–11) are High Affinity Partial Agonists at the Cloned Rat κ-Opioid Receptor. *Eur. J. Pharmacol.* **1997**, *338*, 191–197.

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