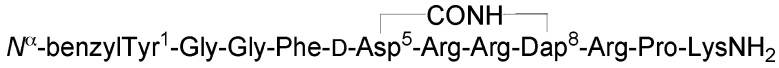
Journal of Medicinal Chemistry

Letter

[*N*-BenzylTyr,*cyclo*(d-Asp,Dap)]- dynorphin A-(1–11)NH Cyclized in the "Address" Domain Is a Novel -Opioid Receptor Antagonist

Kshitij A. Patkar, Xiuzhen Yan, Thomas F. Murray, and Jane V. Aldrich

J. Med. Chem., **2005**, 48 (14), 4500-4503• DOI: 10.1021/jm050105i • Publication Date (Web): 15 June 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Journal of Medicinal Chemistry is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Letters

[N^{α}-BenzylTyr^1,*cyclo*(D-Asp^5,Dap^8)]-dynorphin A-(1−11)NH_2 Cyclized in the "Address" Domain Is a Novel κ-Opioid Receptor Antagonist

Kshitij A. Patkar,[‡,#] Xiuzhen Yan,[§] Thomas F. Murray,[§] and Jane V. Aldrich*[,†]

Department of Pharmaceutical Sciences, School of Pharmacy, University of Maryland, Baltimore, Maryland 21201, Department of Physiology and Pharmacology, College of Veterinary Medicine, University of Georgia, Athens, Georgia 30602, and Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045

Received February 2, 2005

Abstract: The cyclic dynorphin A analogue [N^{α}-benzylTyr^1, *cyclo*(D-Asp^5,Dap^8)]dynorphin A-(1−11)NH_2 (Dap = 2,3-diaminopropionic acid) exhibits nanomolar affinity (30 nM) and high selectivity (K_i ratio (κ/μ/δ) = 1/194/330) for κ-opioid receptors. This analogue antagonizes dynorphin A-(1−13)NH_2 at κ-opioid receptors in the adenylyl cyclase assay (K_B = 84 nM). This is the first dynorphin A-based antagonist with modifications in the C-terminal "address" domain that alter efficacy and thus represents a novel selective κ-opioid receptor antagonist.

Opioid receptors are major targets for the management of pain.[1] Clinically used opiates such as morphine and its analogues exert their analgesic effects predominantly by stimulating μ-opioid receptors.[2] However, the clinical use of these agents for the treatment of chronic pain suffers from severe side effects, most importantly respiratory depression and addiction liability. The complex pharmacology of opioid receptors poses a challenge to the development of analgesics devoid of unwanted side effects. Kappa-opioid receptor ligands have been reported to have a lower tendency to cause respiratory depression and physical dependence than μ-opioid receptor ligands,[3] and hence there is considerable interest in developing κ-opioid receptor ligands as clinically useful analgesics. Recent findings on the analgesic effects of κ-opioid agonists in inflammation have triggered a new interest in developing analgesics that may act through peripheral κ-opioid receptors.[4,5] Several other activities of κ-opioid agonists with potential therapeutic application include anticonvulsant and neuroprotective effects,[6] pharmacological management of cocaine addiction,[7] and the ability to down-regulate HIV-1 expression in human microglial[8] and CD4[9,10] cells. The involvement of κ-opioid receptors in these various activities has made it important to better understand κ-opioid receptors at the molecular level.

Endogenous opioid peptides have been employed as lead compounds in the development of pharmacological tools, including both receptor-selective agonists and antagonists, to study opioid receptors.[2] Dynorphin (Dyn) A is an endogenous κ-opioid receptor agonist.[11] Chavkin and Goldstein proposed the "message-address" concept for Dyn A with the peptide divided into two domains.[11] The N-terminal sequence, which is identical with most other mammalian opioid peptides, was proposed to be the "message" sequence responsible for receptor activation, while the unique C-terminal "address" sequence was postulated to be a potency-enhancing domain for κ-opioid receptors. We have used this concept to design peptide-based antagonists for κ-opioid receptors by modifying the N-terminal "message" sequence.

Antagonists at κ-opioid receptors are important pharmacological tools to study structural and functional characteristics of these receptors. Kappa-opioid receptor antagonists also have potential therapeutic applications in the treatment of opiate addiction[12] and depression.[13] However, only a few Dyn A-based antagonists that are selective for κ-opioid receptors have been reported. We initially reported that the N-terminal acetylated Dyn A analogue JVA-901 (Ac[Lys^2,Trp^{3,4},D-Ala^8]Dyn A-(1−11)NH_2) exhibits antagonist activity at κ-opioid receptors,[14] and we subsequently identified a second acetylated Dyn A analogue arodyn (Ac[Phe$^{1-3}$,Arg^4,D-Ala^8]Dyn A-(1−11)NH_2) with antagonist activity that exhibits higher κ-opioid receptor affinity and selectivity.[15] Schiller and co-workers have demonstrated that replacement of Tyr^1 in opioid peptides, including Dyn A-(1−11)NH_2, by the 2′,6′-dimethyltyrosine derivative in which the positively charged N-terminal amino group is replaced with a methyl group generally converts opioid peptide agonists into antagonists.[16,17] Replacement of Gly in position 3 by Pro has also been reported to impart κ-opioid receptor antagonist activity to the resultant Dyn A-(1−11)NH_2 analogue.[18] However, all of these peptides are linear analogues of Dyn A-(1−11) with modifications in the first four N-terminal residues (the "message" sequence). Only one cyclic Dyn A-(1−11) analogue, the N-terminal cyclic peptide cyclodyn (*cyclo*$^{N\alpha,5}$[Trp^{3,4},Glu^5]Dyn A-(1−11)NH_2) synthesized in our laboratory,[19] has been reported that exhibits antagonist activity.

We were interested in exploring modifications in the C-terminal domain of Dyn A-(1−11) to assess whether such modifications could affect efficacy. Previously we reported that [N^{α}-benzylTyr^1,D-Pro^{10}]Dyn A-(1−11) shows weak agonist activity in the guinea pig ileum (GPI) and partial agonist activity in the adenylyl cyclase assay using cloned κ-opioid receptors expressed on Chinese hamster ovary (CHO) cells.[20−22] Several structure−activity relationship (SAR) studies have shown that a number of modifications in the C-terminal domain of Dyn A-(1−11) are well tolerated without compromising potency or selectivity at κ-opioid receptors.[23] We incor-

* To whom correspondence should be addressed. Tel. (785) 864-2287, fax (785) 864-5326, e-mail jaldrich@ku.edu.
‡ University of Maryland.
§ University of Georgia.
† University of Kansas.
# Current address: Cellular Neurobiology Branch, Cellular Neurophysiology Section, National Institute on Drug Abuse, National Institutes of Health, Baltimore, MD 21224.

10.1021/jm050105i CCC: $30.25
Published on Web 06/15/2005

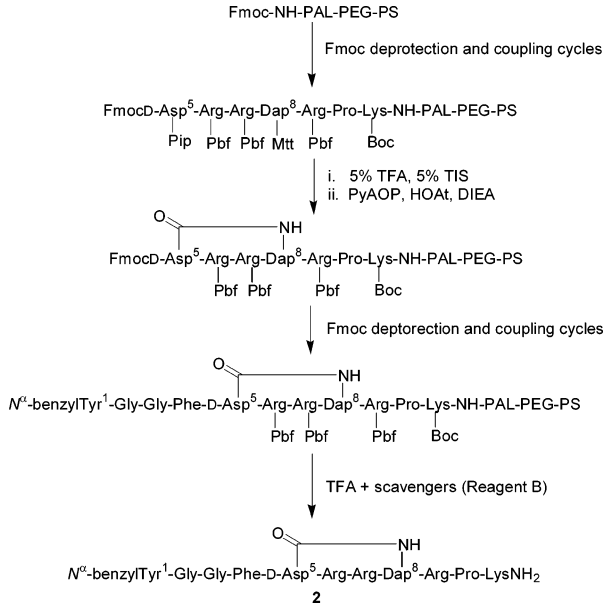
1 N^{α}-benzylTyr^1-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-LysNH$_2$

2 N^{α}-benzylTyr^1-Gly-Gly-Phe-D-Asp^5-Arg-Arg-Dap^8-Arg-Pro-LysNH$_2$ (CONH)

3 N^{α}-benzylTyr^1-Gly-Gly-Phe-D-Asn^5-Arg-Arg-Dap(Ac)^8-Arg-Pro-LysNH$_2$

Figure 1. Structures of cyclic and linear Dyn A analogues.

Scheme 1. Synthesis of Cyclic Peptide **2**



porated several structural modifications in the C-terminal domain of [N^{α}-benzylTyr^1]Dyn A-(1−11) in order to test our hypothesis that C-terminal residues and/or the conformation of the C-terminal domain of Dyn A-(1−11), while not a primary determinant, could affect the efficacy of Dyn A analogues and therefore result in κ-opioid receptor antagonists. Our hypothesis was based on an earlier SAR study of [Phe^1]Dyn A-(1−11)NH$_2$ analogues, where it was reported that C-terminal modifications which had little effect on κ-opioid receptor affinity markedly affected agonist potencies in the GPI and mouse vas deferens (MVD) bioassays.[24]

We incorporated conformational constraints in [N^{α}-benzylTyr^1]Dyn A-(1−11)NH$_2$, **1**, and evaluated their effects on binding affinity and efficacy. Here we describe the results for peptide **2** cyclized between the side chains of D-Asp and Dap (2,3-diaminopropionic acid) in positions 5 and 8, respectively (Figure 1). A linear peptide analogue, **3**, with D-Asn and Dap(Ac) in positions 5 and 8, respectively, was synthesized and evaluated for affinity and efficacy in the same assays to examine the effect of substitution of amino acids in positions 5 and 8 on affinity and efficacy separate from the conformational constraint.

The cyclic peptide **2** was synthesized by the 9-fluorenylmethoxycarbonyl (Fmoc) solid-phase synthetic strategy (Scheme 1). The peptides were assembled on a poly(ethylene glycol)-polystyrene (PEG-PS) resin containing the PAL [peptide amide linker, 5-(4-aminomethyl-3,5-dimethoxyphenoxy)valeric acid] linker generally using 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) and *N*-methylmorpholine as the coupling reagents. The side chain protecting groups on the amino acids used in the syntheses were Pbf (2,2,4,6,7-pentamethyldihydrobenzofurane-5-sufonyl) for Arg and Boc (*tert*-butyloxycarbonyl) for Lys; the hyperacid-labile protecting groups 2-phenylisopropyl (Pip)[25] and 4-methyltrityl (Mtt)[26] were used to protect the side chains of Fmoc-D-Asp and Fmoc-Dap, respectively. The peptide was assembled up through D-Asp^5, and the Pip and Mtt groups were selectively removed using a mixture of 3% trifluoroacetic acid (TFA) and 5% triisopropylsilane (TIS) in dichloromethane. The cyclization between the carboxylic acid of D-Asp and the free amine of Dap was carried out using 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate (PyAOP), 1-hydroxy-7-azabenzotriazole (HOAt), and *N*,*N*-diisopropylethylamine (DIEA) in *N*,*N*-dimethylformamide (DMF). After cyclization, the remaining amino acids, including N^{α}-benzyltyrosine, were coupled to complete the peptide sequence. N^{α}-Benzyltyrosine was synthesized separately in solution by reductive amination of tyrosine *tert*-butyl ester with benzaldehyde using sodium cyanoborohydride in an acidic environment,[27] followed by the deprotection of the *tert*-butyl-protected carboxylic acid using TFA. The linear analogue **3** was synthesized using Fmoc-D-Asn(Trt) (trityl) and Fmoc-Dap(Mtt) in positions 5 and 8, respectively. Acetylation of Dap in position 8 was carried out by selective removal of the Mtt group as described above, followed by acylation using acetic anhydride in DMF. The peptides were cleaved from the resin using Reagent B containing 88% TFA, 5% phenol, 5% H$_2$O, and 2% TIS.[28] The crude peptides were purified to greater than 98% purity by preparative reversed-phase HPLC using a binary solvent system consisting of aqueous 0.1% TFA and acetonitrile containing 0.1% TFA. The peptides were analyzed for purity by reversed-phase HPLC and their molecular weights confirmed by electrospray ionization mass spectrometry.

Table 1. Opioid Receptor Binding Affinities of the Peptides

peptide	$K_i \pm$ SEM (nM)[a] κ	μ	δ	K_i ratio ($\kappa/\mu/\delta$)
1	8.17 ±2.16	84.9 ± 16.5	474 ± 47	1/10/60
2	30.3 ± 1.9	5880 ± 1420	>10000	1/194/>330
3	66.9 ± 5.9	1658 ± 437	>10000	1/25/>142
Dyn A-(1−11)NH$_2$	0.57 ± 0.01	1.85 ± 0.52	6.18 ± 1.01	1/3/11

[a] The binding assays were performed using cloned opioid receptors expressed on CHO cells as described previously[29] using [^{3}H]diprenorphine for κ-, [^{3}H]DAMGO ([D-Ala^2,NMePhe^4,glyol]-enkephalin) for μ-, and [^{3}H]DPDPE (*cyclo*[D-Pen^2,D-Pen^5]enkephalin) for δ-opioid receptors. K_i values are the average ± SEM of 3−6 independent experiments.

In radioligand binding assays using CHO cells stably expressing κ-, μ-, and δ-opioid receptors, the cyclic peptide **2** exhibits nanomolar affinity and high selectivity for κ-opioid receptors (Table 1). The cyclic peptide **2** has 2-fold higher affinity and 8-fold higher selectivity for κ-opioid receptors than the linear peptide **3**. The effect of N$^{\alpha}$-benzylation on κ-opioid receptor affinity varied with the parent peptide. N$^{\alpha}$-Benzylation decreased κ-opioid receptor affinity only about 4-fold for the [D-Asp^5,Dap^8] cyclic peptide (K_i = 30 nM for **2** vs 8.0 nM for *cyclo*[D-Asp^5,Dap^8]Dyn A-(1−13)NH$_2$ prepared previously in our laboratory[29]), while the same modification to other peptides resulted in a much larger decrease in κ-opioid receptor affinity (14-fold lower affinity for **1** vs Dyn A-(1−11)NH$_2$; 94-fold for the corresponding [D-Asp^6,Dap^9] cyclic peptide (data not

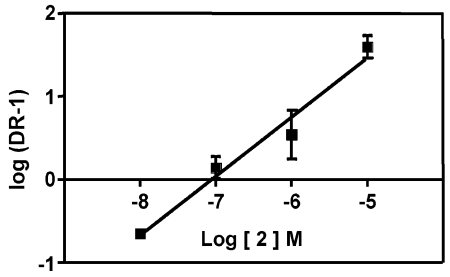


Figure 2. Schild analysis of peptide **2** as an antagonist of Dyn A-(1−13)NH_2 in the adenylyl cyclase assay using CHO cells expressing κ-opioid receptors. DR = dose ratio = EC_{50} of Dyn A-(1−13)NH_2 in the presence of the indicated concentration of **2** divided by the EC_{50} of Dyn A-(1−13)NH_2 in the absence of **2**. Data shown are pooled data from three separate experiments.

shown, K. A. Patkar, T. F. Murray, and J. V. Aldrich, manuscript in preparation)). The functionalities in positions 5 and 8 in both peptides **2** and **3** decrease affinities for μ- and δ-receptors more than for κ-opioid receptors, resulting in enhanced selectivity for κ-opioid receptors compared to the linear peptide **1**. The decrease in affinity was particularly striking for the cyclic peptide **2** at μ-opioid receptors, resulting in exceptional selectivity (194-fold) for κ- over μ-opioid receptors. The low μ-opioid receptor affinity of **2** is a result of combining the N^{α}-benzylTyr[1] modification with the [5,8] cyclization; the cyclic peptide *cyclo*[D-Asp[5],Dap[8]]Dyn A-(1−13)-NH_2 without the N-terminal modification has much higher μ-opioid receptor affinity (K_i = 75 nM) than **2** and only modest selectivity (9-fold) for κ- over μ-opioid receptors.[29]

The C-terminal domain of the peptides has a marked effect on the efficacy observed in the adenylyl cyclase assay using CHO cells stably expressing κ-opioid receptors. The linear parent peptide **1** exhibits 93 ± 5% maximum inhibition of adenylyl cyclase compared to the standard agonist Dyn A-(1−13)NH_2. Other linear [N^{α}-benzylTyr[1]]Dyn A-(1−11)NH_2 analogues modified in the C-terminal sequence exhibit similar efficacy in this assay (73−96% maximum inhibition relative to Dyn A-(1−13)NH_2; K. A. Patkar, T. F. Murray, and J. V. Aldrich, manuscript in preparation). In contrast, the cyclic peptide **2** and its linear counterpart **3** exhibit negligible efficacy (<10% maximum inhibition relative to Dyn A-(1−13)NH_2) in this assay. The cyclic peptide **2** completely reverses the agonist activity of 10 nM Dyn A-(1−13)NH_2 in a concentration-dependent manner (data not shown). Schild analysis[30] of peptide **2** vs Dyn A-(1−13)NH_2 (Figure 2) yielded a K_B of 83.9 nM (8.81−313 nM, 95% confidence interval); the K_B for the nonpeptide antagonist norBNI (nor-binaltorphimine) in this assay is 0.23 nM (0.0013−1.59 nM, 95% confidence interval) as determined by Schild analysis. In the adenylyl cyclase assay using μ- and δ-opioid receptors with DAMGO and DPDPE, respectively, as the agonists, peptide **2** at a concentration of 1 μM did not cause a significant shift in the agonist dose−response curves, indicating that this peptide is a selective κ-opioid receptor antagonist.

These results indicate the importance of both the residues modified and incorporation of the cyclic constraint in determining the κ-opioid receptor affinity, selectivity, and efficacy of these Dyn A analogues. Cyclization to give **2** enhanced κ-opioid receptor affinity 2-fold compared to the linear peptide **3** and increased selectivity for κ- over μ-opioid receptors almost 8-fold due to a combination of an increase in κ- and a decrease in μ-opioid receptor affinities. Modification of positions 5 and 8 in the [N^{α}-benzylTyr[1]] peptides is responsible for the loss of efficacy, with both peptides **2** and **3** exhibiting negligible efficacy at κ-opioid receptors in the adenylyl cyclase assay.

The cyclic peptide **2** is a selective competitive antagonist at κ-opioid receptors and only the second cyclic Dyn A analogue reported that exhibits antagonist activity. It is the first Dyn A-based antagonist with modifications in the "address" domain that alter efficacy at κ-opioid receptors. Thus, this peptide is a novel κ-opioid receptor antagonist that will be an important lead peptide to explore peptide−receptor interactions. We are currently examining the SAR of this lead peptide in order to enhance affinity and antagonist potency at κ-opioid receptors.

Acknowledgment. This research was supported by grant R01 DA05195 from the National Institute on Drug Abuse.

Supporting Information Available: Analytical data (HPLC and mass spectral analysis) for peptides **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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*, 6th ed.; Abraham, D. J., Ed.; John Wiley & Sons: New York, 2003; Vol. 6, pp 329−481.

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

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

(5) 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.

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

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

(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) Lokensgard, J. R.; Gekker, G.; Peterson, P. K. Kappa-opioid Receptor Agonist Inhibition of HIV-1 Envelope Glycoprotein-mediated Membrane Fusion and CXCR4 Expression on CD4-(+) Lymphocytes. *Biochem. Pharmacol.* **2002**, *63*, 1037−1041.

(11) 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.

(12) 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.

(13) Mague, S. D.; Pliakas, A. M.; Todtenkopf, M. S.; Tomasiewicz, H. C.; Zhang, Y.; Stevens, W. C., Jr.; Jones, R. M.; Portoghese, P. S.; Carlezon, W. A., Jr. Antidepressant-like Effects of Kappa-opioid Receptor Antagonists in the Forced Swim Test in Rats. *J. Pharmacol. Exp. Ther.* **2003**, *305*, 323−330.

(14) 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.
(15) Bennett, M. A.; Murray, T. F.; Aldrich, J. V. Identification of Arodyn, a Novel Acetylated Dynorphin A-(1−11) Analogue, as a Kappa Opioid Receptor Antagonist. *J. Med. Chem.* **2002**, *45*, 5617−5619.
(16) Lu, Y.; Nguyen, T. M.; Weltrowska, G.; Berezowska, I.; Lemieux, C.; Chung, N. N.; Schiller, P. W. [2′,6′-Dimethyltyrosine]Dynorphin A(1−11)-NH_2 Analogues Lacking An N-Terminal Amino Group: Potent and Selective Kappa Opioid Antagonists. *J. Med. Chem.* **2001**, *44*, 3048−3053.
(17) Schiller, P. W.; Weltrowska, G.; Nguyen, T. M.; Lemieux, C.; Chung, N. N.; Lu, Y. Conversion of Delta-, Kappa- and Mu-receptor Selective Opioid Peptide Agonists into Delta-, Kappa- and Mu-Selective Antagonists. *Life Sci.* **2003**, *73*, 691−698.
(18) Schlechtingen, G.; Zhang, L.; Maycock, A.; DeHaven, R. N.; Daubert, J. D.; Cassel, J.; Chung, N. N.; Schiller, P. W.; Goodman, M. [Pro^3]Dyn A(1−11)-NH_2: A Dynorphin Analogue with High Selectivity for the Kappa Opioid Receptor. *J. Med. Chem.* **2000**, *43*, 2698−2702.
(19) Vig, B. S.; Murray, T. F.; Aldrich, J. V. 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. *J. Med. Chem.* **2003**, *46*, 1279−1282.
(20) Choi, H.; Murray, T. F.; DeLander, G. E.; Caldwell, V.; Aldrich, J. V. N-Terminal Alkylated Derivatives of [D-Pro^{10}]dynorphin A-(1−11) are Highly Selective for Kappa-opioid Receptors. *J. Med. Chem.* **1992**, *35*, 4638−4639.
(21) Choi, H.; Murray, T. F.; DeLander, G. E.; Schmidt, W. K.; Aldrich, J. V. Synthesis and Opioid Activity of [D-Pro^{10}]-Dynorphin A-(1−11) Analogues with N-Terminal Alkyl Substitution. *J. Med. Chem.* **1997**, *40*, 2733−2739.
(22) Soderstrom, K.; Choi, H.; Berman, F. W.; Aldrich, J. V.; Murray, T. F. N-Alkylated Derivatives of [D-Pro^{10}]Dynorphin A-(1−11) are High Affinity Partial Agonists at the Cloned Rat Kappa-opioid Receptor. *Eur. J. Pharmacol.* **1997**, *338*, 191−197.
(23) Naqvi, T.; Haq, W.; Mathur, K. B. Structure−activity Relationship Studies of Dynorphin A and Related Peptides. *Peptides* **1998**, *19*, 1277−1292.
(24) Kawasaki, A. M.; Knapp, R. J.; Walton, A.; Wire, W. S.; Zalewska, T.; Yamamura, H. I.; Porreca, F.; Burks, T. F.; Hruby, V. J. Syntheses, 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.
(25) 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.
(26) Aletras, A.; Barlos, K.; Gatos, D.; Koutsogianni, S.; Mamos, P. Preparation of the Very Acid-sensitive Fmoc-Lys(Mtt)-OH. Application in the Synthesis of Side-chain to Side-chain Cyclic Peptides and Oligolysine Cores Suitable for the Solid-phase Assembly of MAPs and TASPs. *Int. J. Pept. Protein Res.* **1995**, *45*, 488−496.
(27) Borch, R. F.; Berstein, M. D.; Durst, H. D. Cyanohydridoborate Anion as a Selective Reducing Agent. *J. Am. Chem. Soc.* **1971**, *93*, 2897−2904.
(28) Solé, N. A.; Barany, G. Optimization of Solid-phase Synthesis of [Ala^8]-Dynorphin A. *J. Org. Chem.* **1992**, *57*, 5399−5403.
(29) 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.
(30) Schild, H. O. pA, A New Scale for the Measurement of Drug Antagonism. *Br. J. Pharmacol.* **1947**, *2*, 189−206.

JM050105I