Published in final edited form as:
Tetrahedron Lett. 2006 March 27; 47(13): 2233–2236.

Synthesis of constrained analogues of cholecystokinin/opioid chimeric peptides

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Abstract

In our ongoing research on the synthesis of constrained analogues of CCK/opioid chimeric peptides, a bicyclic dipeptide mimetic for Nle-Asp was designed and synthesized. Starting from β-allyl substituted aspartic acids, the terminal double bond was oxidized resulting in spontaneous cyclization to form racemic hemiaminals. Allylation of the hemiaminals afforded 5-allyl substituted proline analogues, which on oxidation, Horner–Emmons olefination, asymmetric hydrogenation, and bicyclization afforded bicyclic dipeptide mimetics for Nle-Asp. Constrained CCK/opioid peptide analogues containing bicyclic dipeptide mimetics for Nle-Gly, Nle-Asp, and homoPhe-Gly were then synthesized and analyzed at both the CCK and opioid receptors.

Our group has recently been involved in the design and synthesis of chimeric peptides that interact with both the CCK and opioid receptors.[1] Constrained analogues of these peptides have been synthesized via disulfide and lactam cyclizations.[2] To further explore the topographical requirements for interaction of these peptides with receptors, bicyclic dipeptide mimetics for Nle-Gly (**1a**, **1b**, **1c**), and homoPhe-Gly **2** have been designed and synthesized (Fig. 1).[3] In this letter, the design and synthesis of indolizidinone bicyclic dipeptide mimetics for Nle-Asp **3** and the synthesis of peptides containing bicyclic dipeptide mimetics are discussed. The peptides were tested at both the CCK and opioid receptors.

The synthesis of the indolizidinone type of bicyclic dipeptide mimetics has been reported by a number of authors.[4] In our lab, we have developed the synthesis of these mimetics from analogues of pyroglutamic acid.[5] The target compound can be obtained from lactam cyclization of dehydroamino acids derived from Horner–Emmons olefination of allyl substituted proline analogues.

Alkylation of aspartic acid with different electrophiles has been reported by our group[3,6] and other authors.[7] Alkylation with allyl bromide in the presence of lithium bis(trimethylsilyl) amide (LHMDS) and HMPA resulted in the formation of two β-allyl substituted aspartic acids in a total yield of 57% and a ratio of 4:1 in favor of the (2 S, 3R)-**5a** isomer (Scheme 1).

When **5a** was subjected to ozonolysis, the resultant aldehyde spontaneously cyclized to the racemic hemiaminal **6** (Scheme 2). The hydroxyl group was then methylated and the resultant compound **7** reacted with $BF_3 \cdot OEt_2$ and allyl trimethyl silane at −78 °C→rt to give compounds **8a** and **8b** in 48% yield and a ratio of 1:1. When the minor isomer **5b** was subjected to ozonolysis and allylation, only compound **8c** was obtained in 51% yield.

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Supplementary data
Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet. 2006.01.096.

The structure of compound **8a** was confirmed by X-ray crystallography (Fig. 2).

Compound **8a** was then subjected to ozonolysis and the resultant aldehyde subjected to a Horner–Emmons olefination[8] to give the dehydroamino acid **9a** in 58% yield. When osmylation was used for the oxidation of **8a**, the dehydroamino acid was obtained in 71% yield (Scheme 3). Oxidation of compound **8b** by ozonolysis followed by Horner–Emmons olefination gave the desired compound and an unidentified compound in a total yield of 38% and a ratio of 1:1. The reaction was optimized by changing the protocol to oxidation by osmylation followed by olefination to give the desired dehydroamino acid **9b** in 35% yield.

Osmylation and Horner–Emmons olefination of **8c** gave the dehydroamino acid **9c** in 40% yield. Asymmetric hydrogenation of the dehydroamino acids **9a**, **9b**, and **9c** gave the saturated analogues, which after deprotection of the Boc group were cyclized by heating in pyridine at 50 °C for 4 days to give the bicyclic dipeptide mimetics **3a**, **3b**, and **3c**, respectively.

The three bicyclic dipeptide mimetics were then characterized by NOE measurements (Fig. 3). A strong NOE value was observed for H^3 and H^6 in compounds **3b** and **3c** (4.2% and 4.0%, respectively) signifying a cis relationship between the two hydrogens. For compound **3a**, the NOE value between H^3 and H^6 was relatively low (1.9%) due to the trans relationship between the hydrogens. The cis relationship between H^8 and H^9 in **3a** and **3b** resulted in a strong NOE value of 4.8% compared with the weak value of 1.9% observed for compound **3c**.

To introduce the bicyclic dipeptide mimetics **1a**, **1b**, **1c**, and **2** into the peptides, the methyl ester was hydrolyzed and the N^{α}-Boc group deprotected using TFA. The amino group was then Fmoc-protected. For compound **3a**, both the carboxyl and amino groups were deprotected by hydrogenation and the amino group Fmoc-protected. Fmoc/*t*-Bu solid phase peptide synthesis method was used for the synthesis of the peptides. The peptides **JMN1-5** (Fig. 4) were consequently synthesized and analyzed at both the CCK and opioid receptors.

When the peptides were evaluated at the opioid receptors they showed weak activity at both the δ- and μ-opioid receptors (Table 1). Peptide **JMN2**, however, showed low micromolar binding affinity for both the δ- and μ-opioid receptors. The only peptide that showed an agonist effect at the opioid receptors is peptide **JMN5** though it had low binding affinity.

At the CCK receptors, peptides **JMN1-4** showed low micromolar binding affinities and biological activities at the CCK-B receptors (Table 2). This can be attributed to the C-terminal tetrapeptide (Trp-Nle-Asp-Phe), which has been shown to be the minimum sequence required for activity at CCK-B receptors. Introduction of a bicyclic dipeptide mimetic for Nle-Asp (see **JMN6**) led to loss of activity at the CCK receptors possibly due to interference with the tetrapeptide unit. Substitution of D-Trp for Trp in **JMN4** to give **JMN5** led to loss of activity at the CCK receptors even though there was improved activity at the opioid receptors. However, unlike in most other CCK peptides where substitution of D-Trp leads to antagonistic properties,[1] **JMN5** retained agonist properties. The peptides had relatively low binding profile at the CCK-A receptors and showed no agonist biological activity.

In conclusion, novel bicyclic dipeptide mimetic containing CCK/opioid chimeric peptides was synthesized and evaluated at both the CCK and opioid receptors. Peptides **JMN1-5** were active at the CCK-B receptors while **JMN5** that contained a D-Trp showed weak opioid activity. To discover peptides that will have agonist properties at the opioid receptors and antagonist properties at the CCK-B receptors, more analogues of these peptides will need to be synthesized. Our first target would be the substitution of D-Trp for Trp on all the peptides, which may lead to improved opioid activity as with **JMN5**. A combination of D-Trp^4 and $NMeNle^5$ may also lead to improved activities at both the opioid and CCK receptors and possibly this may impart CCK antagonistic properties.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work is supported by grants from the US Public Health Service DA 12394 and DA 06284.

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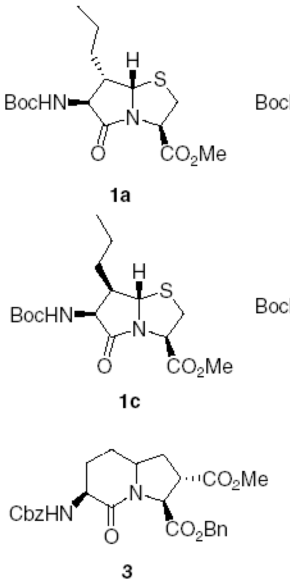


Figure 1.
Bicyclic dipeptide mimetics.

Figure 2.
X-ray crystal structure of compound **8a**.

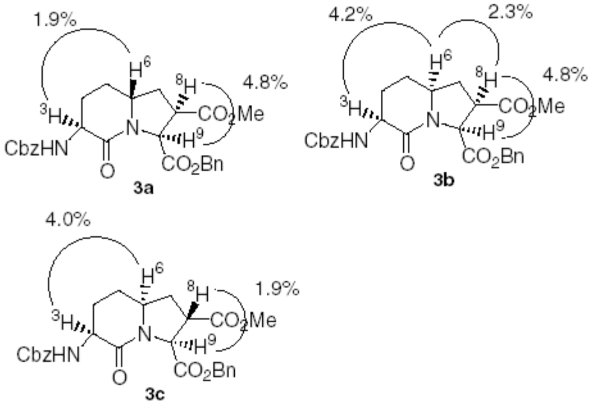
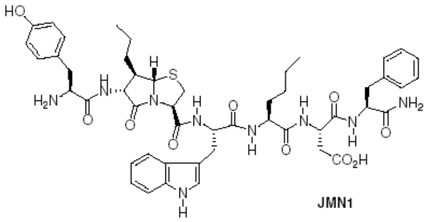
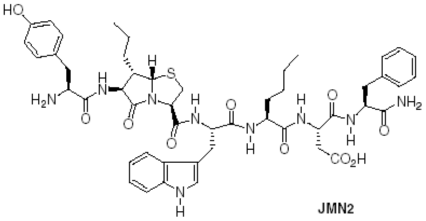


Figure 3.

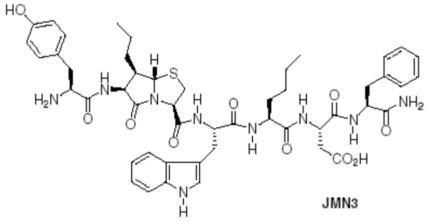
NOE data for Nle-Asp bicyclic dipeptide mimetics.



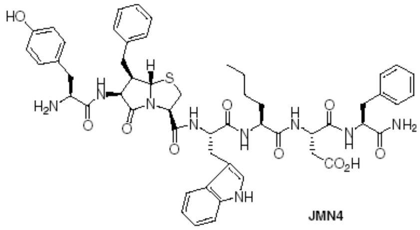
JMN1



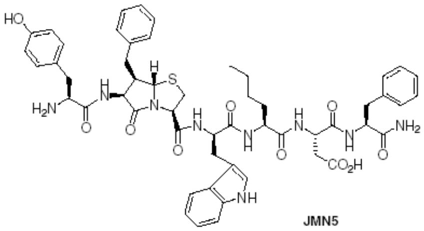
JMN2



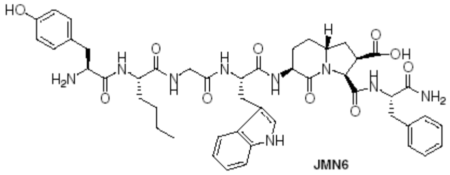
JMN3



JMN4



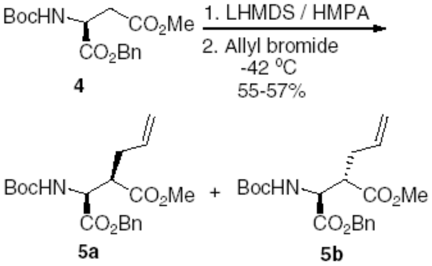
JMN5



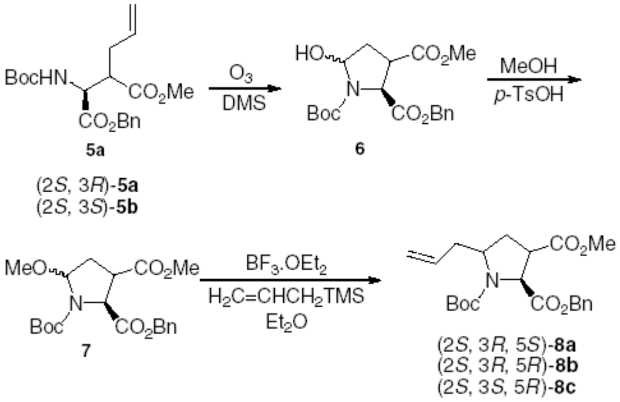
JMN6

Figure 4.

Novel bicyclic dipeptide mimetic containing CCK/opioid chimeric peptides.

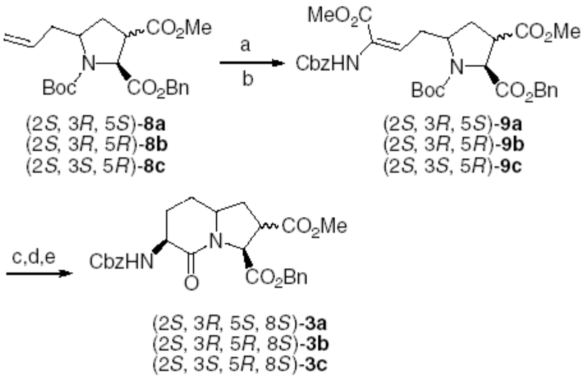


Scheme 1.
Alkylation of aspartic acid with allyl bromide.



Scheme 2.

Synthesis of δ-allyl-substituted proline analogues.



Scheme 3.

Synthesis of Nle-Gly bicyclic dipeptide mimetic. Reagents and conditions: (a) OsO_4, $NaIO_4$; (b) $(MeO)_2POCH(NHCbz)CO_2Me$, DBU; (c) (*S*,*S*)-COD-EtDUPHOSRh(I)OTf, H_2; (d) 20% TFA/DCM, (e) pyridine, 50 °C.

Table 1

Biological evaluation at the opioid receptors

	GTP binding[a]		Competition[b]	
Drug	**hDOR EC$_{50}$ (nM)**	**rMOR EC$_{50}$ (nM)**	**hDOR IC$_{50}$ (nM)**	**rMOR IC$_{50}$ (nM)**
SNF9007	n/d	n/d	250	5200
RSA501	1000	1800	74	1000
JMN1	n/d	n/d	1850	9300
JMN2	NA	NA	337	220
JMN3	NA	NA	1700	3500
JMN4	NA	NA	1400	8600
JMN5	460	770	2700	8100
JMN6	NA	880	1500	3100

n/d = not determined, NA = no activity at 10^{-5} M.

[a] [^{35}S]GTP-γ-S binding assay.

[b] Competitive binding assays against radiolabeled [^{3}H]DPDPE at hDOR and [^{3}H]DAMGO at rMOR. hDOR and rMOR were expressed from CHO cell lines.

Table 2

Biological evaluation at CCK receptors

	Functional analysis[a]		Binding affinity[b] (k_i, nM)		
Drug	**hCCK-A EC_{50} (nM)**	**hCCK-B EC_{50} (nM)**	**hCCK-A [^{125}I] CCK_8**	**hCCK-B [^{125}I] CCK_8**	**CCK-A agonist activity[c]**
SNF9007	n/d	n/d	3300	2.1	n/d
RSA501	790	3100	140	14	None
JMN1	NA	4.1	740	70	None
JMN2	NA	6.9	1200	100	None
JMN3	NA	2.3	2400	16	None
JMN4	NA	1.9	810	11	None
JMN5	NA	160	1800	1400	None
JMN6	NA	NA	>10,000	>10,000	None

n/d = not determined, NA = no activity at 10^{-5} M.

[a] Phosphoinositide (PI) hydrolysis assay in hCCK-A and hCCK-B receptors in HEK cell lines.

[b] Competition against [^{125}I] CCK_8 (sulfated) in hCCK-A and hCCK-B receptors in HEK cell lines in the presence of naloxone.

[c] Contraction of isolated tissue relative to initial contraction with KCl in the presence of naloxone in GPI/LMMP.