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Heptapeptide Mimic of Ohmefentanyl Binding in the Discontinuous μ-Opiod Receptor†

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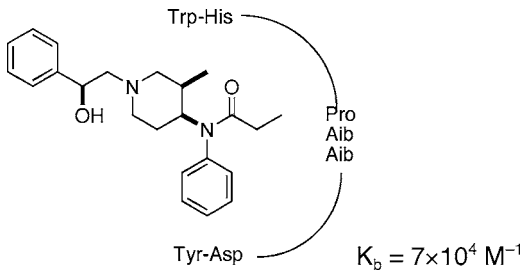
ABSTRACT



Ohmefentanyl binds to the rat μ-opiod receptor via two dipeptide sequences (Trp-His and Asp-Tyr) that are separated by 170 residues. A turn-inducing tripeptide, Pro-Aib-Aib, holds the dipeptides in a conformation that binds the narcotic ($K_b = 7.1 \times 10^4\ M^{-1}$) in THF. Binding is specific for ohmefentanyl over morphine and is accompanied by a conformational change in the heptapeptide host. Control experiments with a Gly-Gly-Gly tripeptide linking the dipeptides show no evidence of binding.

Small molecules specifically bind to proteins for any number of reasons, both dynamically and statically. Substrates enter an active site of an enzyme, become more tightly bound through a transition state, and then dissociate. Drugs bind to receptors and elicit a change in protein conformation and/or activity. Small-molecule toxins may bind to sites in a similar way to a drug, precipitating a change in protein conformation or function, or they may bind to orphan receptors, for which there is no known endogenous ligand or substrate. Site-directed mutagenesis, X-ray crystallography, and molecular modeling are able to define, in a very precise manner, the nature of the interactions between the protein and a bound small molecule. Often, the residues that comprise a binding site are discontinuous. Considerable effort has been spent in the past decade to mimic discontinuous protein receptors[1] and, more generally, to discover new types of host−guest interactions[2] using combinatorial techniques.

An obvious approach to the design of biomimetic receptors is to use combinatorially designed peptides. To arrange the side chains in a three-dimensional array around a small-molecule guest, most such approaches employ a parallel arrangement of peptides on a scaffold. For example, steroids,[3]

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† Dedicated to Professor Richard G. Hiskey, on the occasion of his 76th birthday.

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(1) A discontinuous binding site is one where the pertinent residues are distant in the amino acid sequence and brought into spatial proximity by protein folding. See: Eichler, J. *Prot. Pept. Lett.* **2004**, *11*, 281−290.

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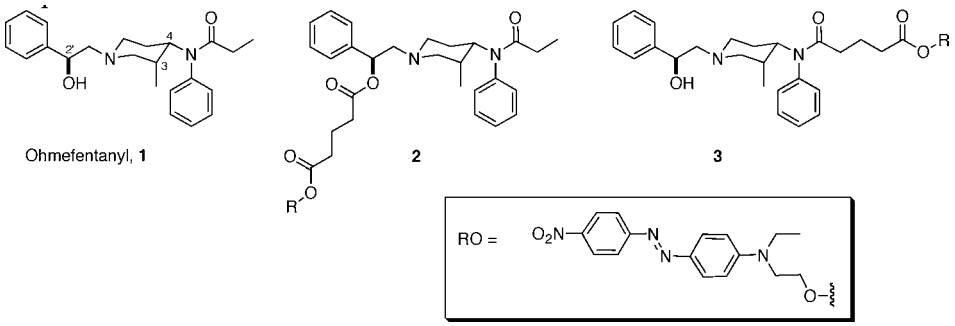


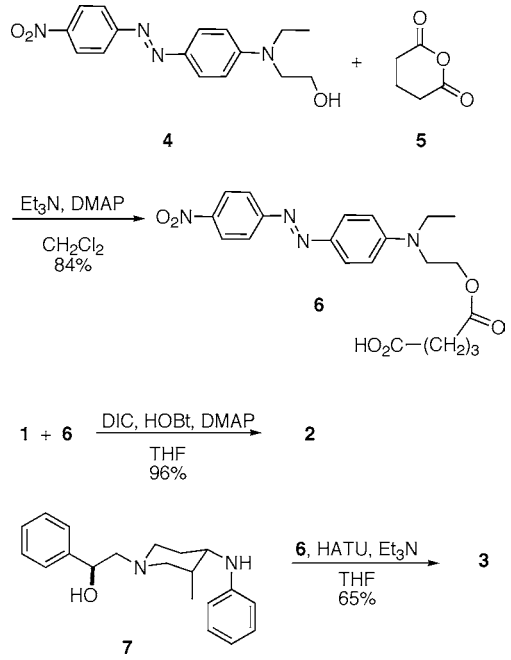
Figure 1. 2′*S*,3*R*,4*S*-Ohmefentanyl and two dyed derivatives.

cyclic peptides,[4] azacyclophanes,[5] and diketopiperazines[6] have been used to direct peptides in a parallel arrangement in 3D space. Although there is a considerable body of work describing the structural features of β- ($i/i + 3$ H-bonds) and γ-turns ($i/i + 2$ H-bonds) in the design of peptide mimetics,[7] such studies are largely directed at turning the peptide chain in a manner that propagates secondary structure, not to facilitate ligand binding. We are not aware of any previous studies in which a short sequence of amino acids is used to effect a turn to replace a scaffold.

To investigate such a possibility, we chose to begin with a mimic of the binding of the narcotic 2′*S*,3*R*,4*S*-ohmefentanyl (**1**; Figure 1), to the rat μ-opiod receptor. In this system, $Asp_{147}Tyr_{148}$ and $Trp_{318}His_{319}$ have been implicated in the binding of the narcotic.[8] To restate the issue in the context of the binding of ohmefentanyl to the μ-opiod receptor: can the 170-residue chain separating these two critical dipeptide binding domains be replaced by as few as three amino acids? There is an important secondary question as well: can a peptide that is barely long enough to possess any secondary structure provide the necessary conformational preorganization to act as a receptor for a small molecule?

To test for binding of ohmefentanyl to a library of peptide receptors, the narcotic (prepared by literature procedures[9]) was derivatized with a red dye in two places, attached via a hemiglutarate, to afford narcotic-dye conjugates **2** and **3**. The synthesis of **2** and **3** is outlined in Scheme 1. Red disperse

Scheme 1



dye (**4**) was acylated with glutaric anhydride (**5**) in 84% yield to give the dye-acid **6**. Coupling of the latter to ohmefentanyl afforded **2**, and coupling to *N*-norpropionyl ohmefentanyl[9] (**7**) afforded **3** (see the Supporting Information for experimental details).

Our first attempt at an ohmefentanyl binding peptide was a library of six heptapeptides containing one each of Leu (L), Asn (N), and Aib (B, α-aminoisobutyric acid) separating the two critical dipeptides: WH_ _ _DY. The thinking was that the Aib would encourage a turn, and since they have been implicated in binding,[10] perhaps the Leu and Asn would aid binding in this short sequence. The library was synthe-

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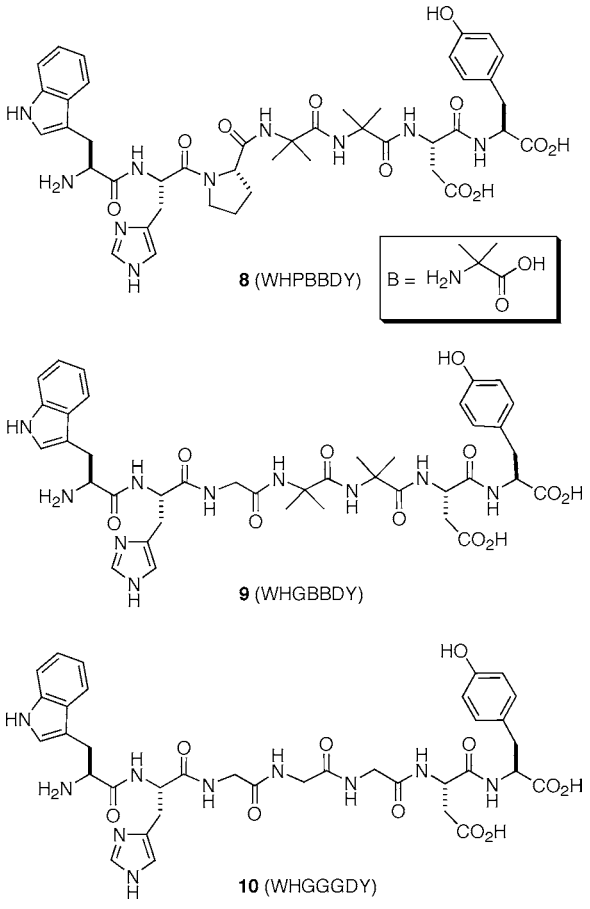
sized in parallel on the solid phase, but when it was tested against the dyed narcotics **2** and **3**, no specific binding could be discerned. We decided to try a tripeptide that more strongly encouraged a turn, while allowing for either positive or negative ϕ/ψ torsion angles. Therefore, we prepared a parallel 27-member library (WH_ _ _DY) containing Pro (P) and Aib (B) as turn-inducing residues, along with Gly (G) to impart flexibility. A Pro-Aib sequence has been shown to induce a β-turn in the design of chiral, peptidic acylation catalysts.[11] Since Aib and Gly are achiral, there is no intrinsic positive or negative ϕ/ψ angle bias. Details of the library synthesis may be found in the Supporting Information.

Samples of about five beads from each library member were incubated, with gentle stirring, for 2 days in 10 mL of 9.7×10^{-5} M solutions of **2** and **3**, in both THF and $CHCl_3$ (see the Supporting Information). No discernible color differences could be observed in the beads soaked in the THF and $CHCl_3$ solutions of **3** or in the $CHCl_3$ solution of **2**. However, in THF, two library members produced a strong color with **2**: WHPBBDY (**8**) and WHGBBDY (**9**). Several



other peptides, each of which contained at least two of the turn-inducing residues P and B, showed faint color. Of these, **8** appeared to show the strongest binding and was chosen for further investigation, along with WHGGGDY (**10**), which showed no binding in the bead assay.

To ensure that peptide **8** was indeed binding to ohmefentanyl and not the dye,[12] **8** was synthesized on the solid phase (see the Supporting Information). A fluorescence titration, irradiating the tryptophan residue of **8** at 280 nm, revealed the binding isotherm shown in Figure 2. A small amount of

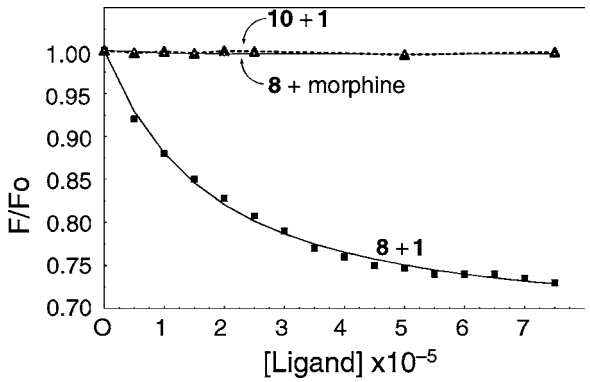


Figure 2. Binding isotherms for titration of **1** against **8** and **10** and for morphine against **8**. Conditions: [**8** or **10**] = 5×10^{-6} M; λ_{irr} = 280 nm; emission integrated from 290 to 500 nm.

water in the THF was necessary to dissolve the peptide. Nonlinear least-squares analysis of the hyperbolic curve[13] revealed a binding constant of 7.1×10^4 M^{-1}. Peptide **10**, in which three glycines separate the WH and DY dipeptides, was also synthesized on the solid phase. Titration of **10** with **1** showed no evidence of binding (Figure 2). Selectivity of the binding of heptapeptide **8** to ohmefentanyl **1** was demonstrated by a titration of **8** with morphine, in which no evidence of binding was seen (Figure 2).

To probe possible changes in the secondary structure of **8** in the presence and absence of **1**, the CD spectra shown in Figure 3 were recorded. Although **1** showed no circular dichroism, peptide **8** showed a positive Cotton effect at 230 nm, which broadened and intensified in the presence of an equimolar concentration of **1**. We interpret this as evidence of a conformational change upon binding of **1** to **8**. A similar

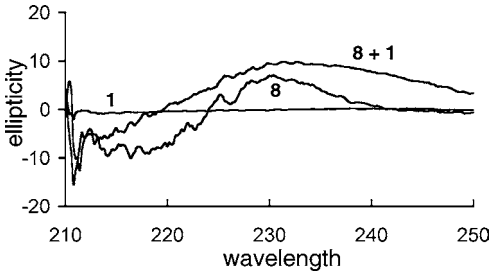


Figure 3. Circular dichroism spectra of **1** (5×10^{-4} M), **8** (5×10^{-4} M), and **1** + **8** (each 2.5×10^{-4} M in moist THF).

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experiment with **8** and morphine showed no change in the CD spectrum of **8**, consistent with the lack of interaction of **8** with morphine. Although details of the conformation of the peptide and the mode of binding must await further study, it is clear that heptapeptide **8** has sufficient preorganization to allow recognition of **1**, while also accommodating the narcotic with a conformational change. The lack of binding of **1** to **10** confirms the long-held notion that preorganization of a receptor is a prerequisite for binding. The fact that a conformational change is also observed in the binding of **1** to **8** indicates that the types of conformational changes observed in proteins upon ligand binding may also be observed in much smaller peptide hosts.

In summary, we have shown that, in THF solution, a turn-inducing tripeptide can hold the four critical residues of the μ-opiod receptor in a conformation that binds the narcotic ohmefentanyl. The binding of **1** to **8** is 3−4 orders of magnitude lower than the binding of **1** to the μ-opiod receptor. Although the nature of the turn induced by the PBB sequence is not known at this stage, a conformational change is evident upon binding, and the binding is selective for ohmefentanyl over morphine. Since the μ-opiod receptor is a membrane-bound protein, the binding site is buried in the lipid bilayer and is in a relatively nonpolar environment. The moist THF solvent used in this study may mimic the relatively hydrophobic environment of the protein/membrane binding site. It seems likely that the two Aib residues help induce a turn by torsional constraints; the aprotic THF solvent may also permit hydrogen bonding to further restrict conformational motion. Further studies on these aspects are in progress and will be reported in due course.

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Supporting Information Available: Details of the synthesis of **2**, **3**, **6**, **8** and **10**, the peptide libraries, the incubation studies, and NMR spectra of **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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