# Bivalent Mu-Delta Opioid Ligands: Potential for Pain Control Without Tolerance or Dependence

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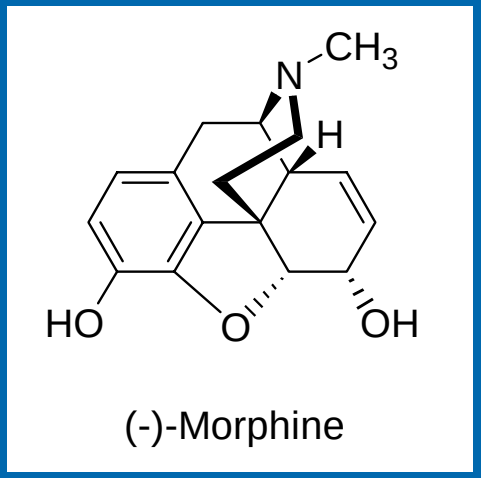
# Opioid Analgesics



(-)-Morphine

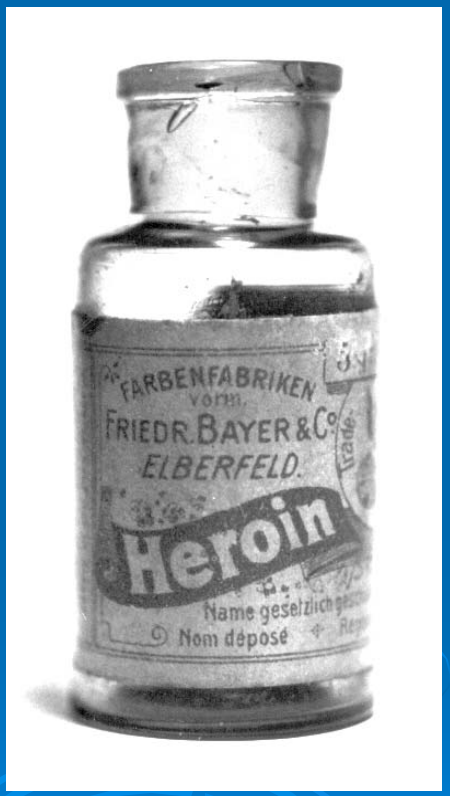
## Opium

- From the poppy Papaver Somniferum
- One of the oldest recorded medications
- Long history of use and abuse

- Morphine: Isolated from opium poppy seeds in the early 1800s.
- Morpheus (latin) = Greek God of Dreams

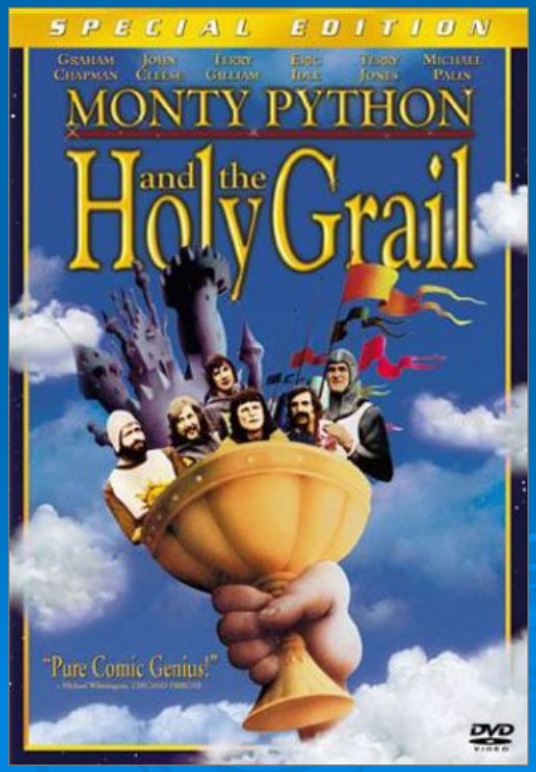
# Diacetylmorphine - Heroin

- 1st Synthesized in 1874 by C.R. Alder Wright
- 1897 - Bayer Pharmaceutical company "re-invented" diacetylmorphine
  - named it **Heroin** after the German word heroisch → "heroic"
  - 1898 – 1910 Bayer marketed Heroin as a non-addictive morphine substitute
- 1914 Harrison Narcotics Tax Act
- 1924 US banned all use of Heroin

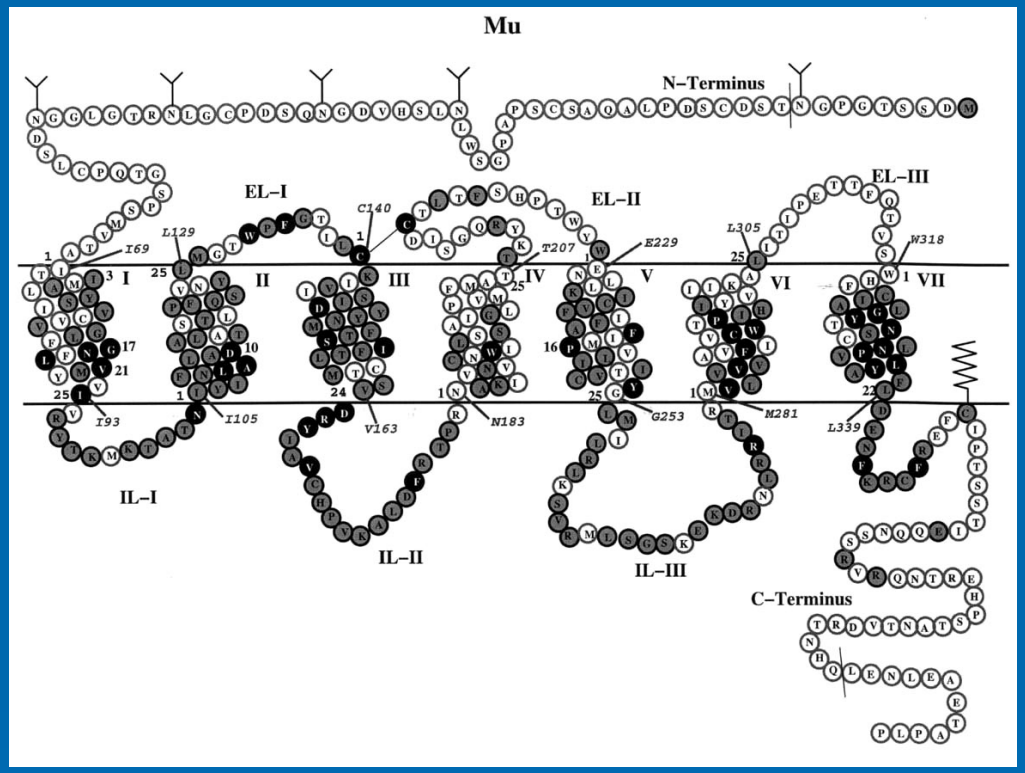


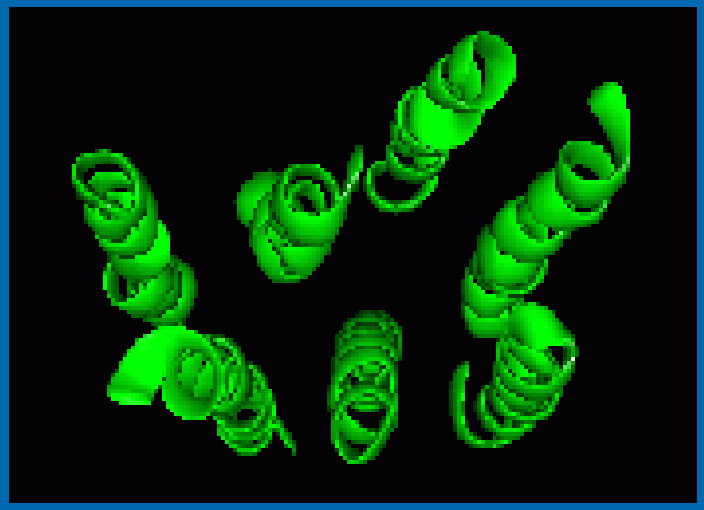
# Opioid Analgesics – The Holy Grail

- Countless synthetic opiate compounds have been synthesized over the last century in an attempt to create the “perfect” analgesic



# History and Background



- G protein-coupled receptors
- 3 Opioid Receptor Subtypes:  Mu, Delta, Kappa
- Approximately 60 % homology
- 372 to 398 amino acid residues

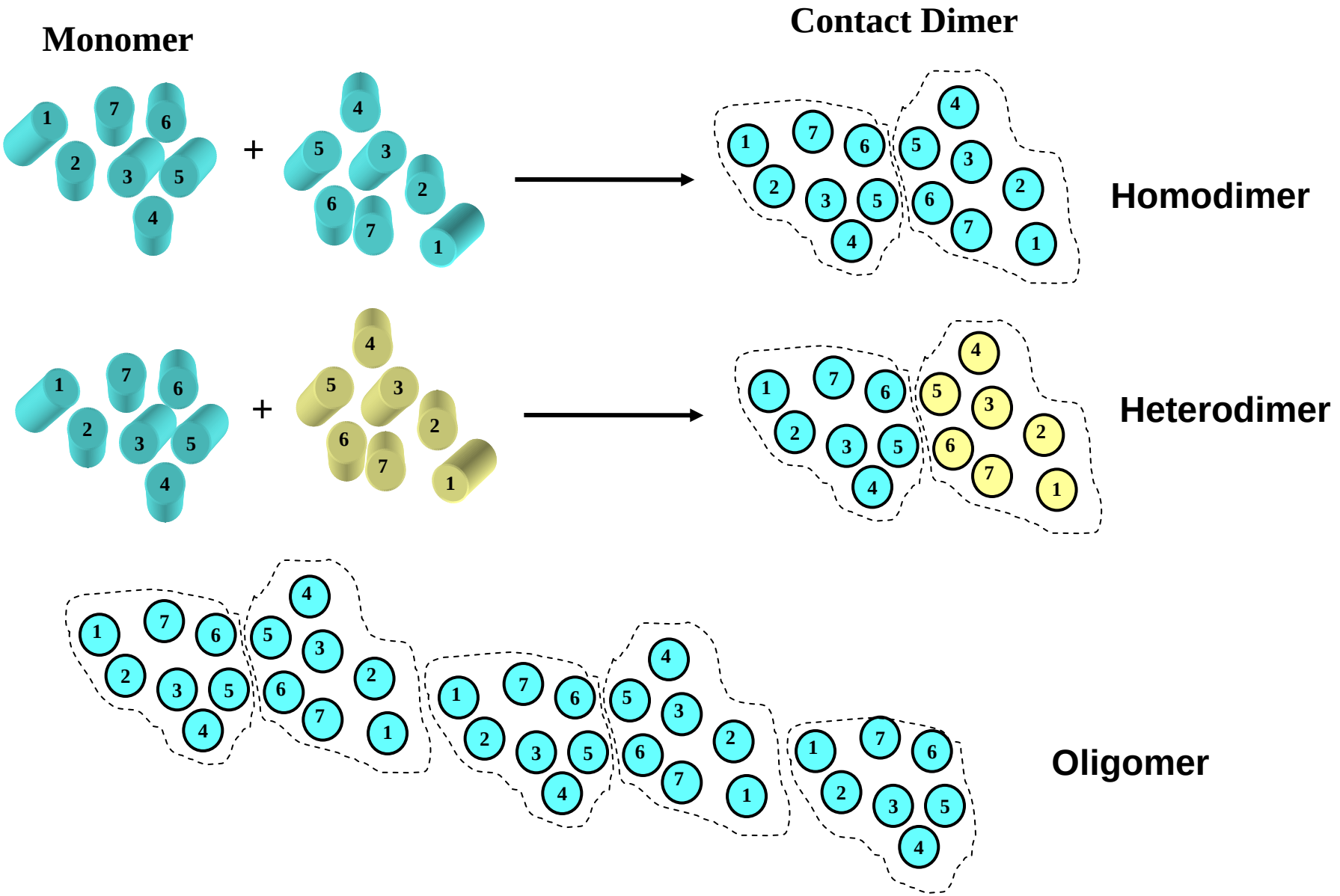
# Opioid Receptor Subtypes

$Mu_1$, $Mu_2$, $Mu_3$
$Delta_1$, $Delta_2$
$Kappa_1$, $Kappa_2$

- Pharmacology predicts greater receptor subtypes than receptor cloning reveals!
- Single gene-deleted mice lack all subtypes for deleted receptor

Opioid Receptor Dimerization/Oligomerization?

# GPCR Dimerization



# Opioid Receptor Dimerization

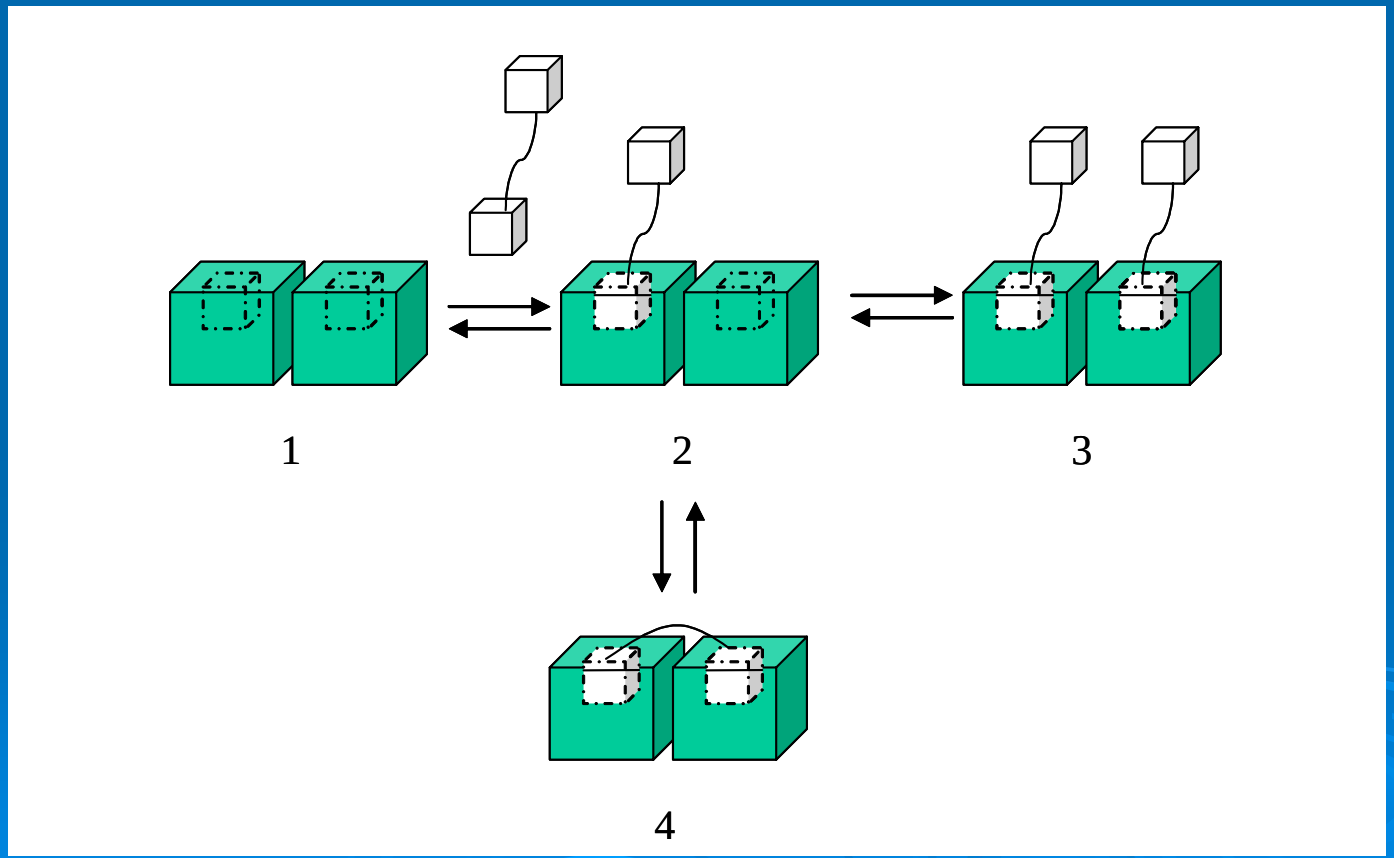
- Homodimers:
  - Mu-Mu
  - Delta-Delta
  - Kappa-Kappa
- Heterodimers:
  - Mu-Delta
  - Delta-Kappa
  - Mu-Kappa

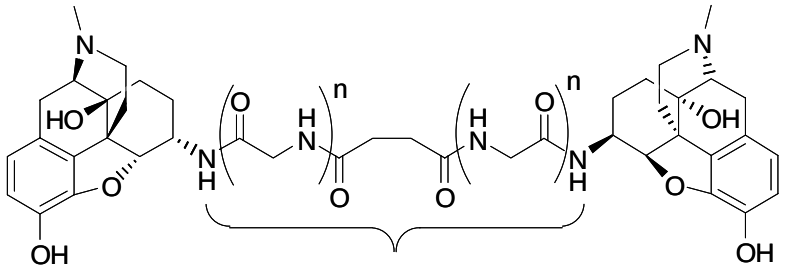
Evidence for dimerization

1. Pharmacological
2. Coimmunoprecipitation
3. BRET and FRET
4. Confocal microscopy
5. Atomic-force microscopy

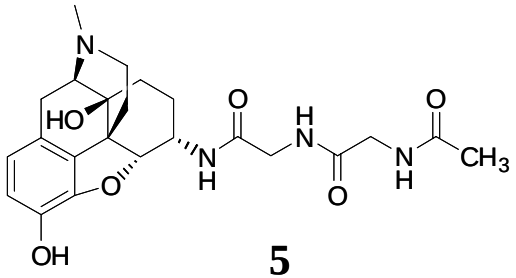
# Bivalent Ligands

- Pharmacological Probes Selective for Dimerized Opioid Receptors



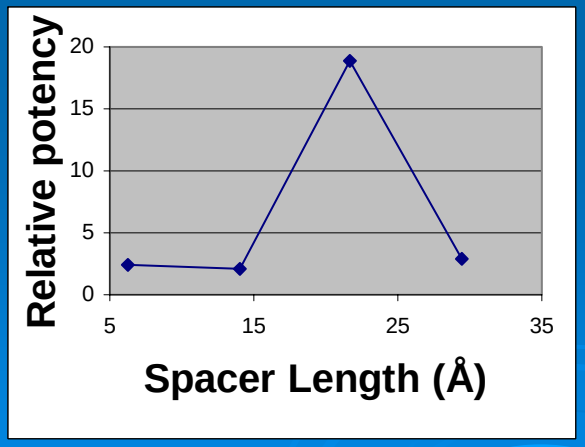


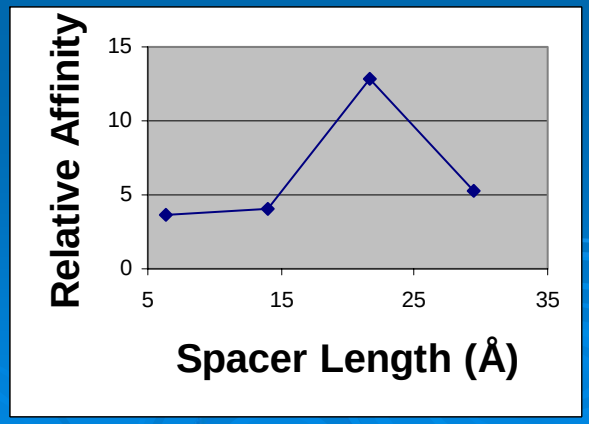

Spacer Length




**5**

| Cmpd no. | n | Spacer Length[a] Å | $IC_{50}$ nM | Rel potency[b] |
|---|---|---|---|---|
| **1** | **0** | **6.3** | **30.2 ± 8.7** | **2.4** |
| **2** | **1** | **14.0** | **22.0 ± 5.0** | **2.1** |
| **3** | **2** | **21.7** | **3.9 ± 0.6** | **18.9** |
| **4** | **3** | **29.4** | **25.1 ± 5.2** | **2.9** |
| **5** | | | **66.8 ± 11.7** | **1.0** |




Portoghese, P.S. et. al. *Journal of Medicinal Chemistry*, **1986**, 29, 1855-1861

# Purpose of our Projects

- Develop selective bivalent ligands to probe opioid receptor organization
- See if we can characterize putative opioid receptor subtypes
- Gain additional insight into the molecular organization of opioid receptors
- **Develop novel analgesics devoid of tolerance and physical dependence**

# Early Evidence For Interactions Between Mu and Delta Opioid Receptors

- Potentiation of morphine with delta agonists
  - Vaught, J.; Takemori, A. Differential effects of leucine enkephalin and methionine enkephalin on morphine-induced analgesia, acute tolerance and dependence. *J. Pharm. Exp. Ther.*, **1979**, 208, 86-94
  - Rothman,R.B.; Westfall,T.C. Allosteric coupling between morphine and enkephalin receptors in vitro. *Mol Pharmacol*., **1982**, 21, 548-557

# Pharmacological Evidence for Tolerance and Dependence

- Abdelhamid, E.E.; Sultana, M.; Portoghese, P.S.; Takemori, A.E. Selective blockage of delta opioid receptors prevents the development of morphine tolerance and dependence in mice. *J. Pharm. Exp. Ther.,* **1991**, 258, 299-303

- Zhu, Y.; King, M.A.; Schuller, A.G.; Nitsche, J.F.; Reidl, M.; Elde, R.P.; Unterwald, E.; Pasternak, G.W.; Pintar, J.E. Retention of supraspinal morphine-like analgesia and loss of morphine tolerance in delta opioid receptor knockout mice. *Neuron,* **1999** 24, 243-252

# Recent Evidence For Interactions Between Mu and Delta Opioid Receptors

- Cultured cells
  - George, S.R.; Fan, T.; Xie Z.; Tse R.; Tam, V.; Varghese, G.; O'Dowd, B.F. Oligomerization of mu and delta-Opioid Receptors. *Journal of Biological Chemistry,* **2000***,* 275, 26128-26135
  - Gomes, I.; Jordan, B.A.; Gupta, A.; Trapaidze, N.; Nagy, V.; Devi, L.A. Heterodimerization of mu and delta Opioid Receptors: A Role in Opiate Synergy, *The Journal of Neuroscience*, **2000**, 20, 1-5
- Living Cells - BRET
  - Mu luciferase and delta YFP cotransfected into living cells
- Spinal cord membranes
  - Coimmunoprecipitation experiments with mouse abs to mouse mu and mouse delta opioid receptors in WT and delta "knockout" mice

    Gomes, I.; Gupta, A.; Filipovska, J.; Szeto, H. H.; Pintar, J. E.; Devi, L. A. A role for heterodimerization of mu and delta opiate receptors in enhancing morphine analgesia. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, 101, 5135-5139.

# Mu-Delta Bivalent Ligand Project

**M D A N - #**

**MU**

**Delta**

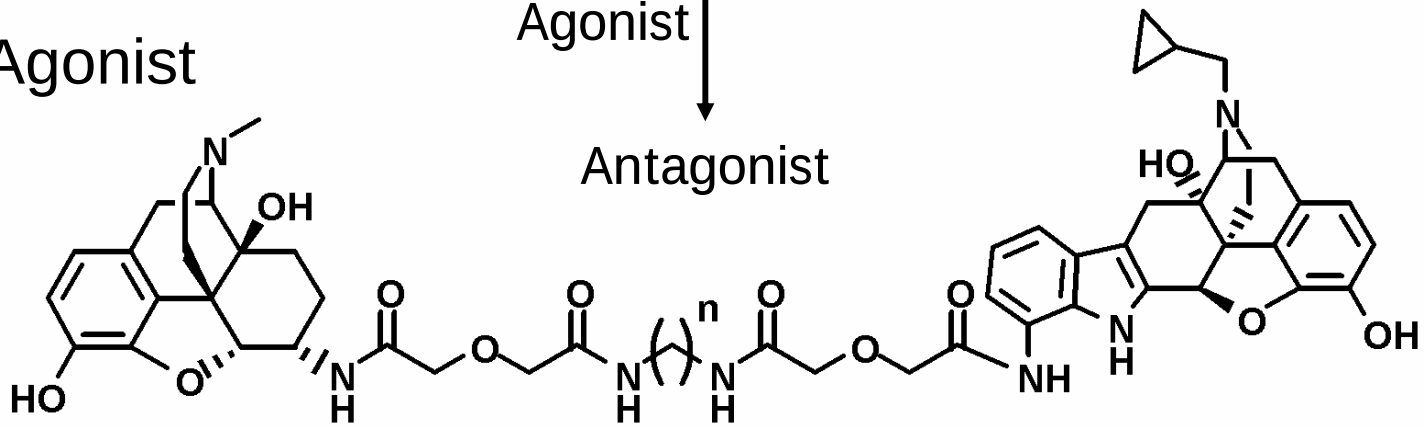
Agonist

Antagonist

# of Atoms of the spacer

μ – Agonist

δ – Antagonist

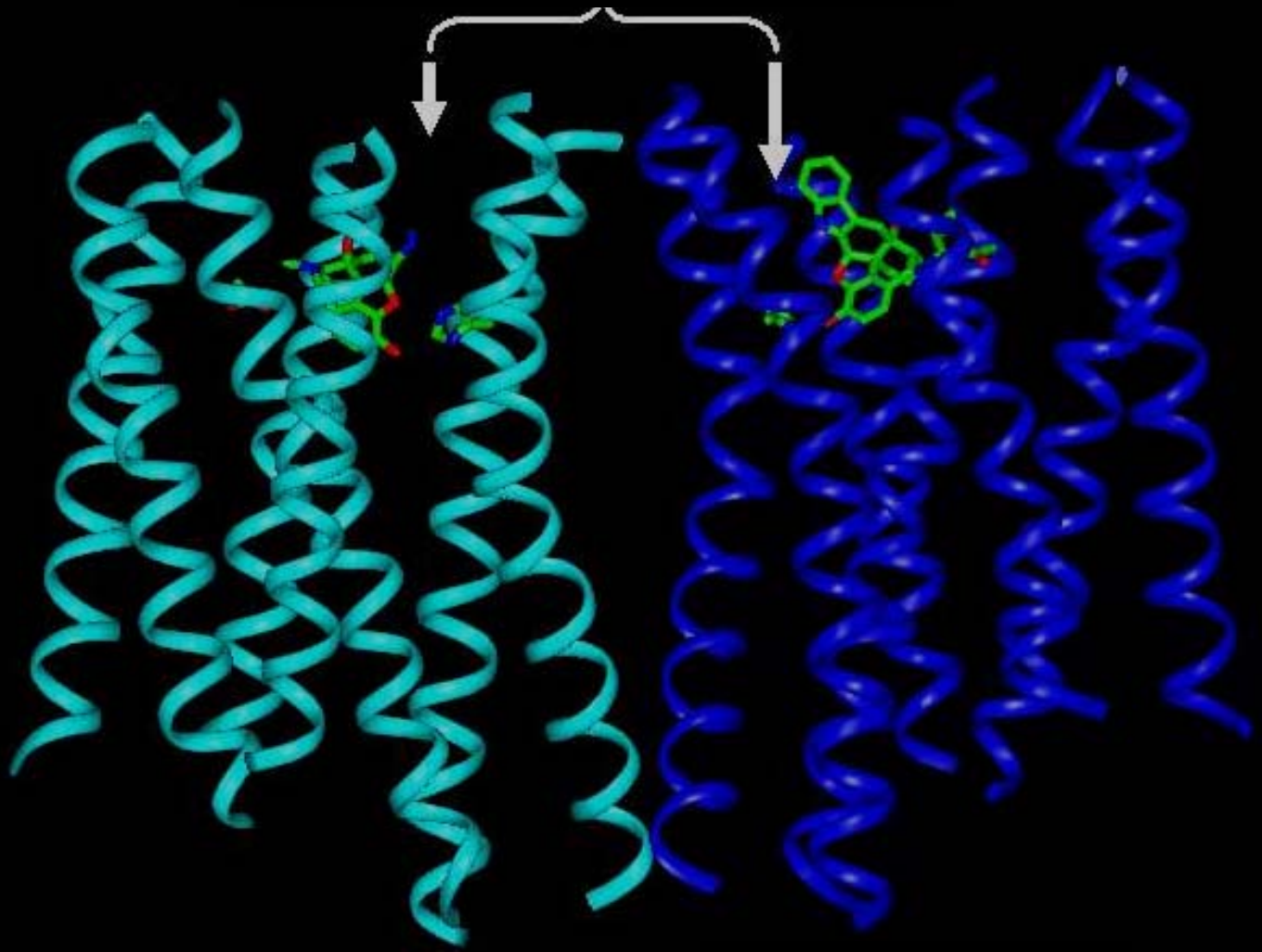



Daniels, D.J.; Lenard, N.R.; Etienne, C.L.; Law, P.Y.; Roerig, S.C.; Portoghese, P.S. Opioid-induced tolerance and dependence in mice is modulated by the distance between pharmacophores in a bivalent ligand series. *Proc. Natl. Acad. Sci. U.S.A.* **2005,** 102, 19208-19213**.**

# Model of a Mu-Delta Heterodimer

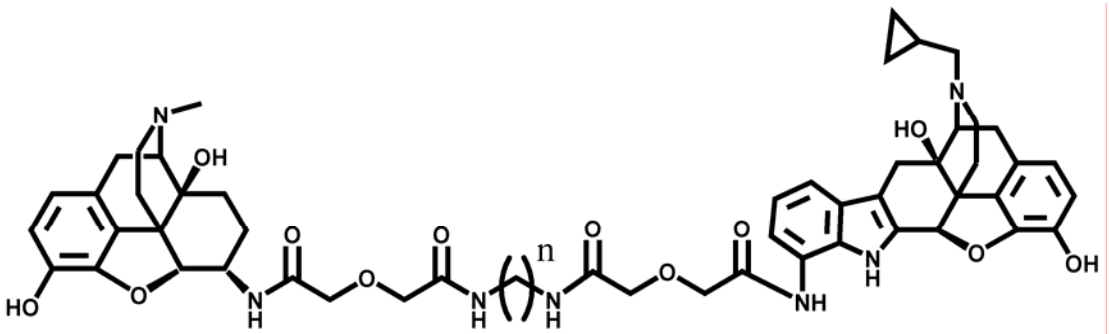
MDAN-19 through MDAN-21 Spacer Distance = 23 -26 Å

**Approximate Distance Between recognition sites = 17 Å**

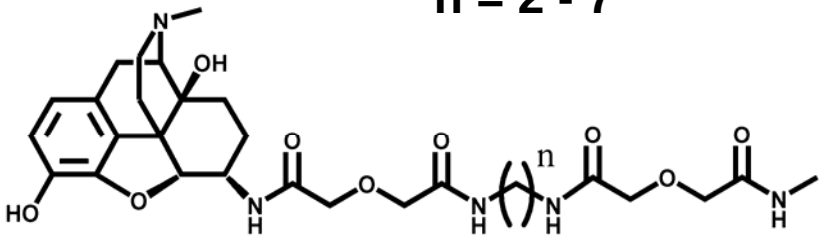


**Mu Receptor** **Delta Receptor**

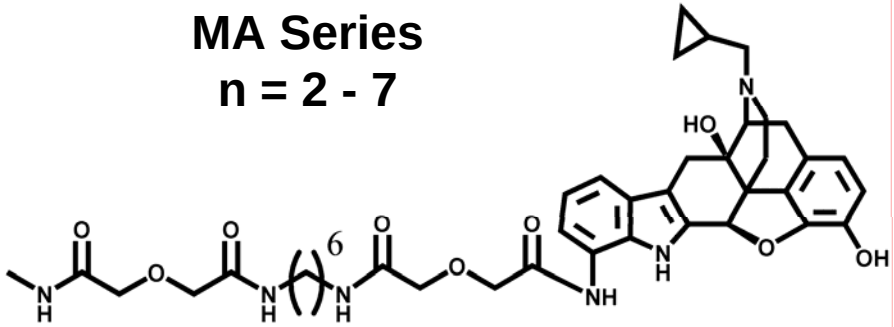
# Mu-Delta Bivalent Ligand Project



**MDAN Series**
**n = 2 - 7**



**MA Series**
**n = 2 - 7**

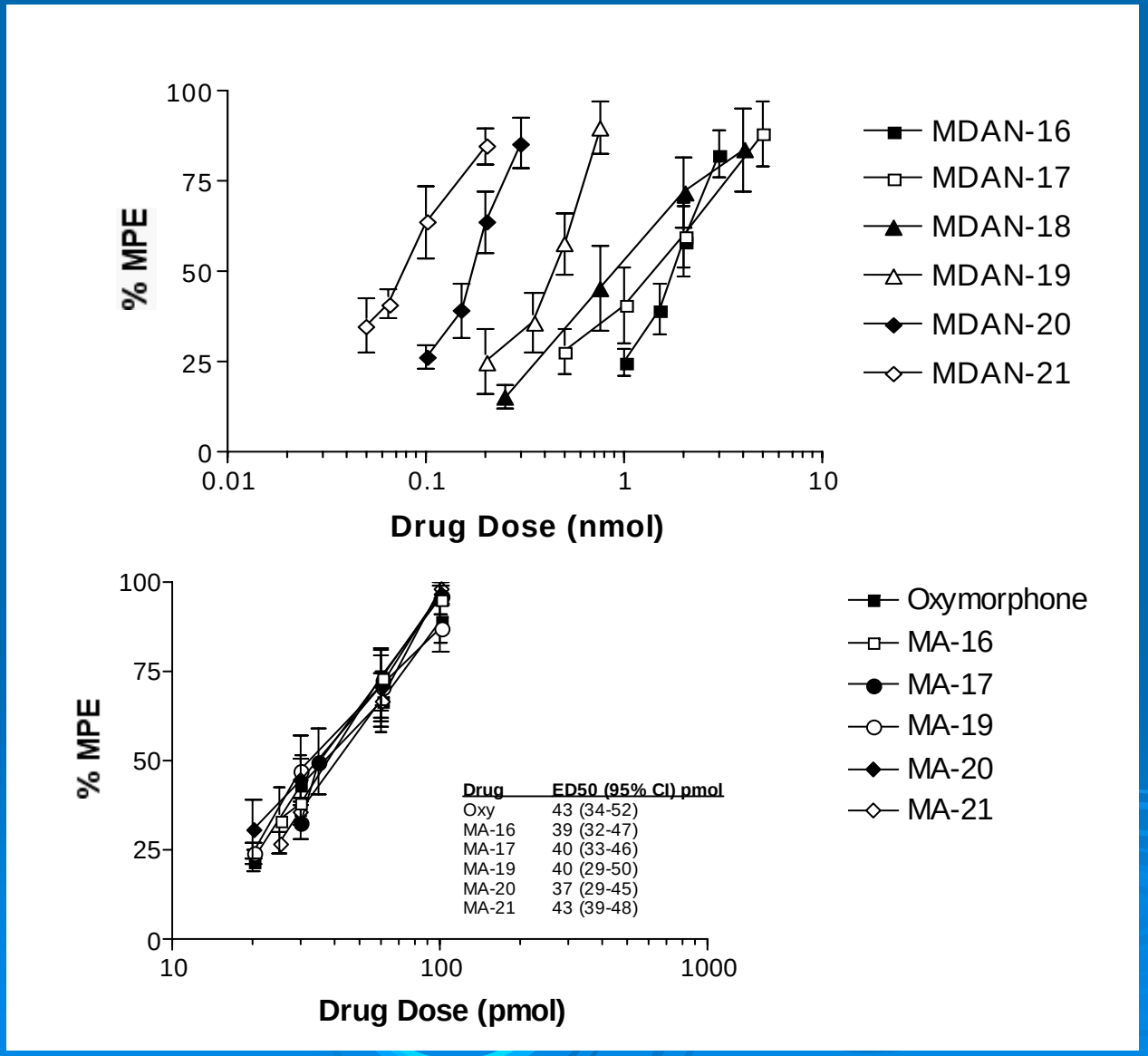


**DN-20**

# Measure of analgesia/antinociception


**Mouse Tail Flick Assay**

# Antinociceptive activity in the mouse tail-flick assay after acute I.C.V administration



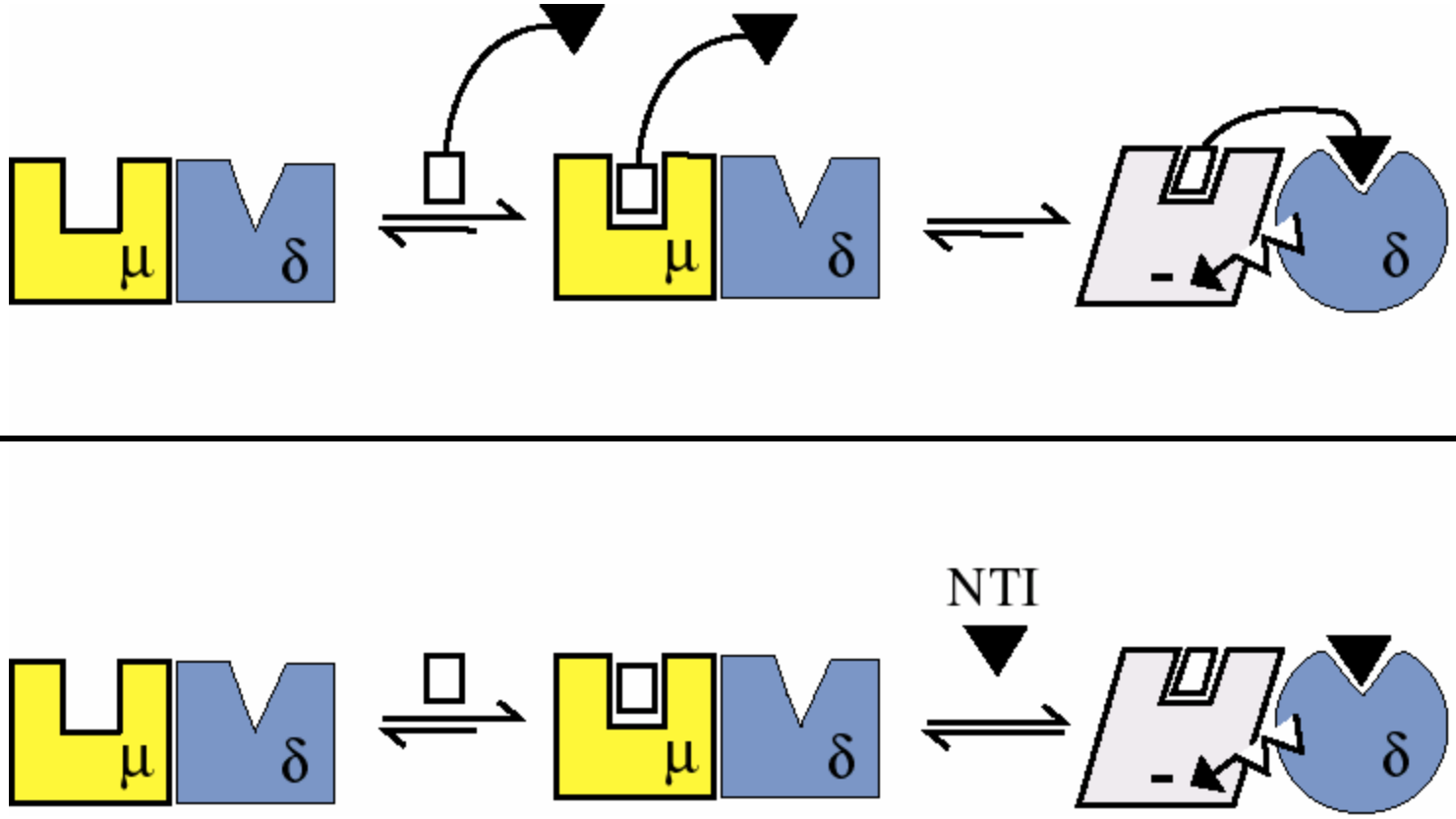
# Model for Testing Tolerance and Physical Dependence in Mice

- Osmotic minipump infuses constant rate of ligand via cannula i.c.v. for 3 days
- Day 4, naloxone (1 mg/kg s.c.) injected; the number of jumps in ten minutes is recorded
- 4 hours laters: “chronic” ED50 value with tail-flick assay
- Tolerance determined by comparing saline infusion vs. chronic infusion

# Effect of Spacer Length on Tolerance and Dependence in Mice

| | Tolerance | | | Dependence |
|---|---|---|---|---|
| Compound | Saline $ED_{50}$ nmol (95% C.I.) | Chronic $ED_{50}$ nmol (95% C.I.) | Fold Diff | # of Jumps (SEM) |
| MDAN - 16 | 1.62 (1.35 – 1.89) | 4.72 (3.47 – 5.91) | 2.8 | 30 (23) |
| MDAN - 17 | 1.54 (0.89 – 2.20) | 5.61 (4.39 – 6.83) | 3.6 | 0.9 (0.7) |
| MDAN - 18 | 1.29 (0.97 – 1.61) | 4.75 (3.50 – 6.00) | 3.7 | 8.9 (3.0) |
| **MDAN - 19** | **0.42 (0.37 – 0.47)** | **0.40 (0.33 – 0.47)** | **1.0** | **3.6 (1.7)** |
| **MDAN - 20** | **0.17 (0.15 – 0.20)** | **0.17 (0.13 – 0.21)** | **1.0** | **0.4 (0.4)** |
| **MDAN - 21** | **0.10 (0.09 – 0.11)** | **0.10 (0.09 – 0.11)** | **1.0** | **3.5 (1.7)** |
| MA - 19 | 0.04 (0.03 – 0.05) | 0.22 (0.19 – 0.26) | 5.5 | 83 (13) |
| MA-19 + DN-19 | 0.04 (0.03 – 0.04) | 0.03 (0.02 – 0.05) | 9.4 | 29 (8) |
| **Morphine** | **4.54 (3.51 – 5.56)** | **26.8 (20.8 – 32.8)** | **6.0** | **100 (15)** |

# Model for the role of μ–δ heterodimers in tolerance and dependence



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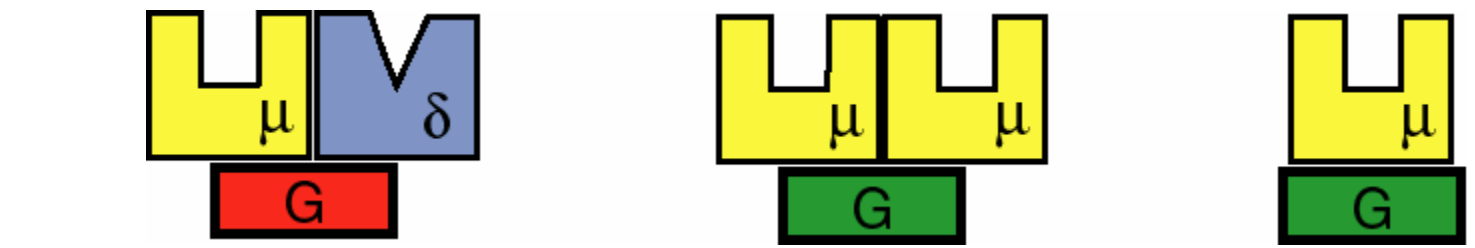



**CAMKII ??**

**Tolerance & Dependence**

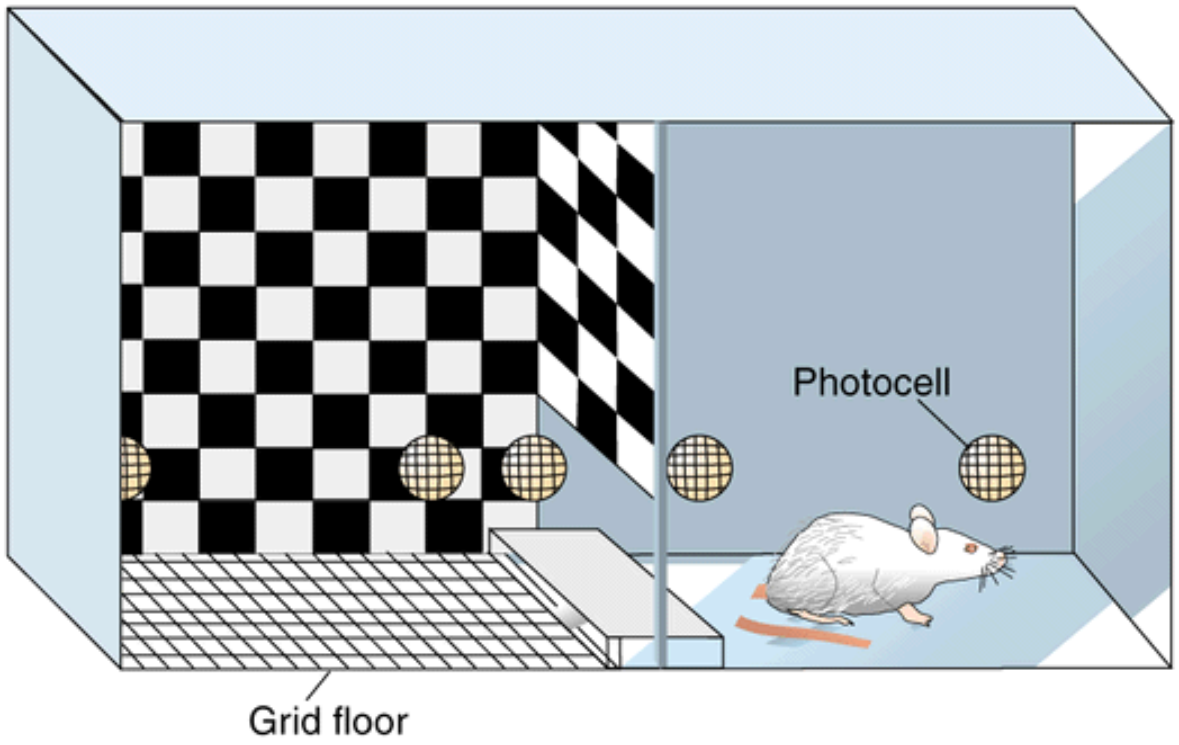
**Analgesia**

**Analgesia**

**Analgesia**

**Opioid receptor organization may be responsible for separate mechanisms leading to tolerance, dependence, and other unwanted side effects**

# Conditioned Place Preference
# A Model of Addiction




Adapted from Feldman, R.S.; Meyer, J.S. and Quenzer, L.F. Principles of Neuropsychopharmacology Sunderland, MA, Sinauer Associates, 1997.

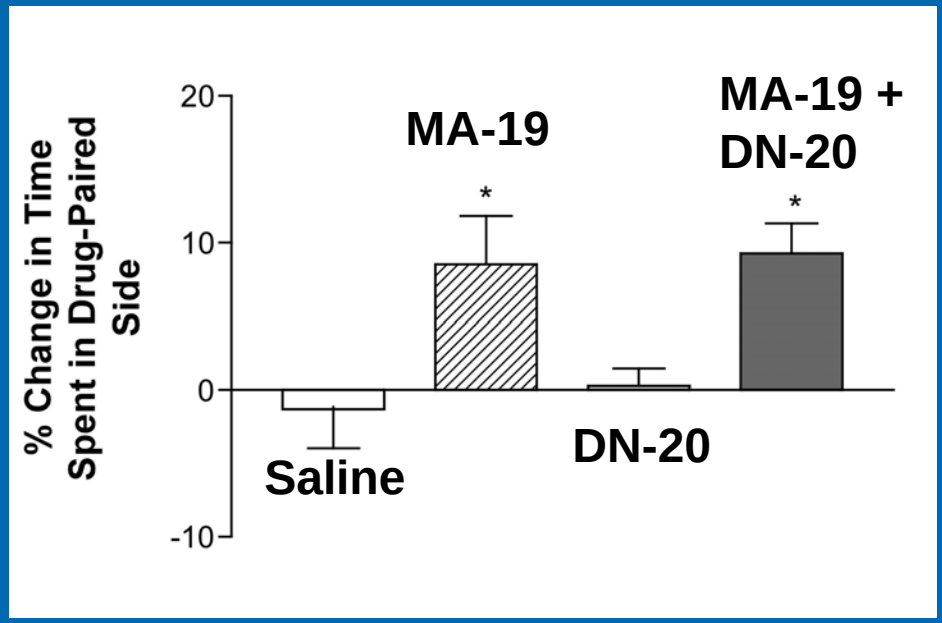
Lenard, N.R.; Daniels, D.J.; Portoghese, P.S.; Roerig, S.C. Absence of conditioned place preference or reinstatement with bivalent ligands containing mu-opioid agonist and delta-opioid receptor antagonist pharmacophores. *Eur. J. Pharmacol.* **2007**, Accepted and in press.

# Acquisition of Place Preference

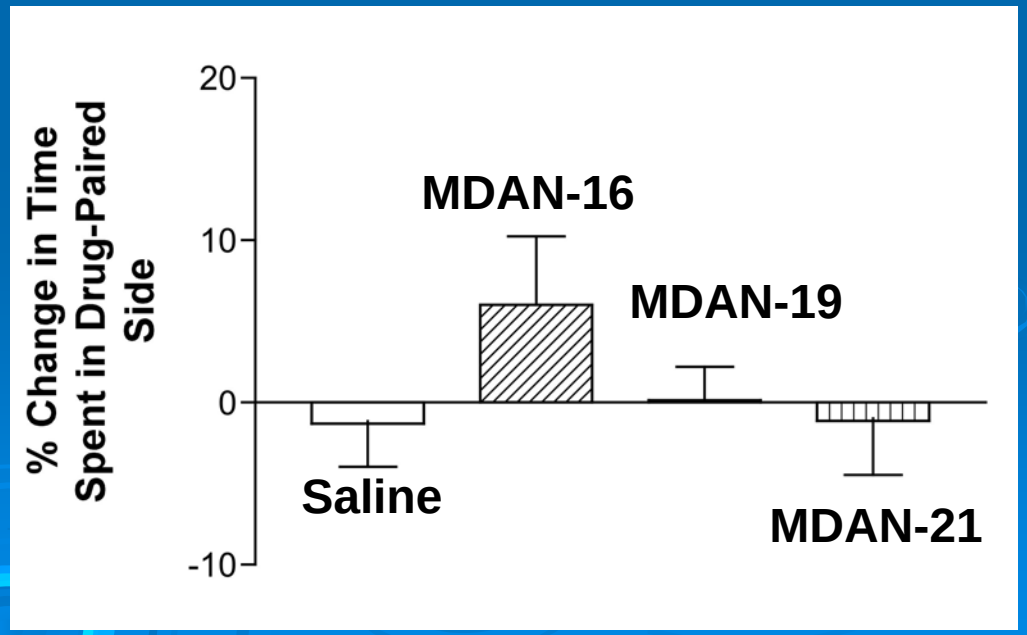
- Day 1: Exposure to novel environment (15 min)
- Day 2: Preconditioning
  - The time spent in each side of the box in 15 min recorded
- Days 3 – 5: Injected with saline and confined to one side of box (30 min); Later injected with ligand and confined to other side of box (30 min)
- Day 6: Determine place preference (15 min)
  - Place Preference = Percent change in time spent on drug-paired side
  - Positive = Place Preference
  - Negative = Place Aversion

# Conditioned Place Preference Results

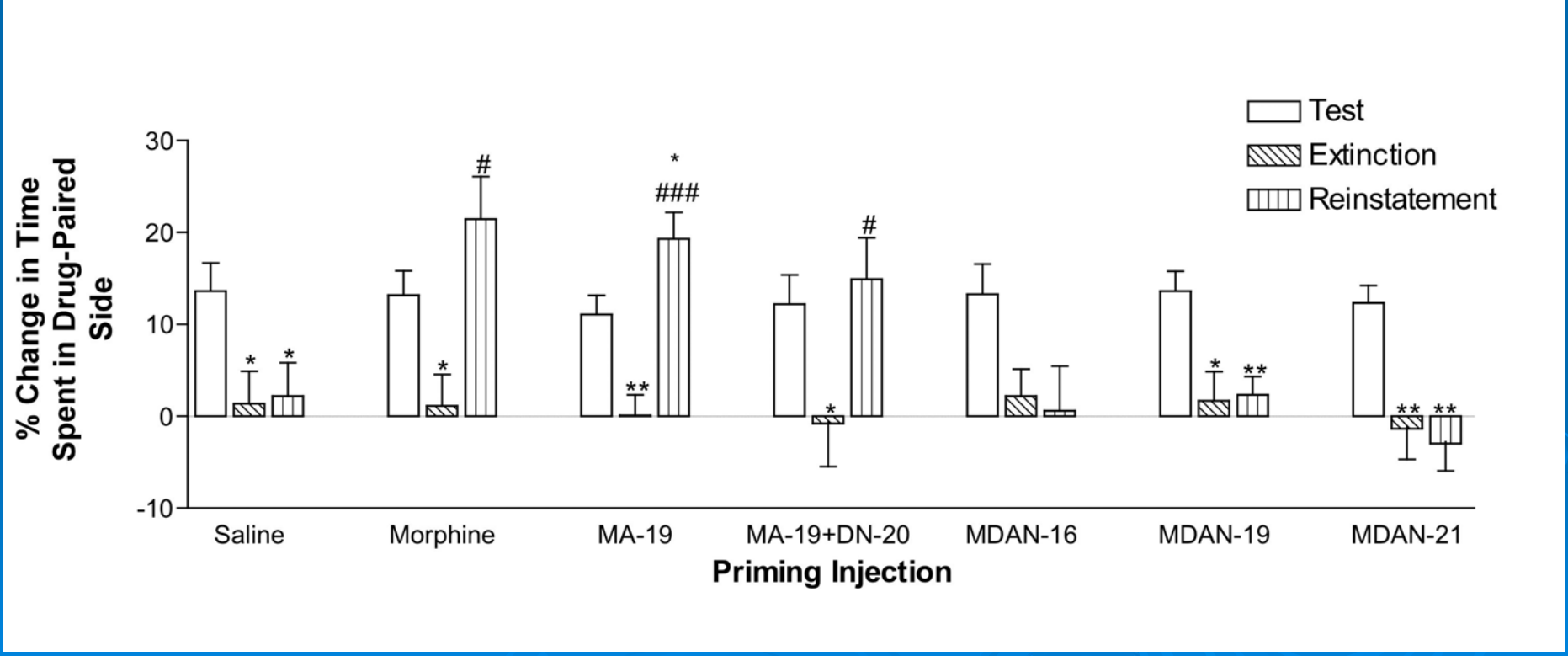
**Monovalent Ligands**




**Bivalent Ligands**



# Primining injection-induced reinstatement of morphine CPP



# Parenteral Bioavailability

**Comparison of Intravenous to Intracerebral Ventricular Administration Potencies for MDAN-21, MA-19 and Morphine**

| Ligand | i.c.v. $ED_{50}$ (95% C.I.) nmol | i.v. $ED_{50}$ (95% C.I.) nmol | i.v. / i.c.v. ratio |
|---|---|---|---|
| **MA-19** | 0.04 (0.03 – 0.05) | 1.61 (1.29 – 1.92) | **40.3** |
| **MDAN-21** | 0.08 (0.06 – 0.10) | 3.3 (3.0 – 3.6) | **41.3** |
| Morphine | 4.1 (3.7 – 4.8) | 168 (146 – 178) | **41.0** |

# Project Summary

- Clinical Implications
  - Potent, efficacious analgesics
  - No tolerance
  - No physical dependence
  - No drug seeking behavior
- Molecular Implications
  - Tolerance and dependence mediated through associated mu-delta opioid receptors??

# Future Directions

- University of Minnesota is in the process of patenting the MDAN series
- Several pharmaceutical companies have expressed interest

- Other laboratories are in the process of designing compounds with mixed μ-agonist/δ-antagontist activity

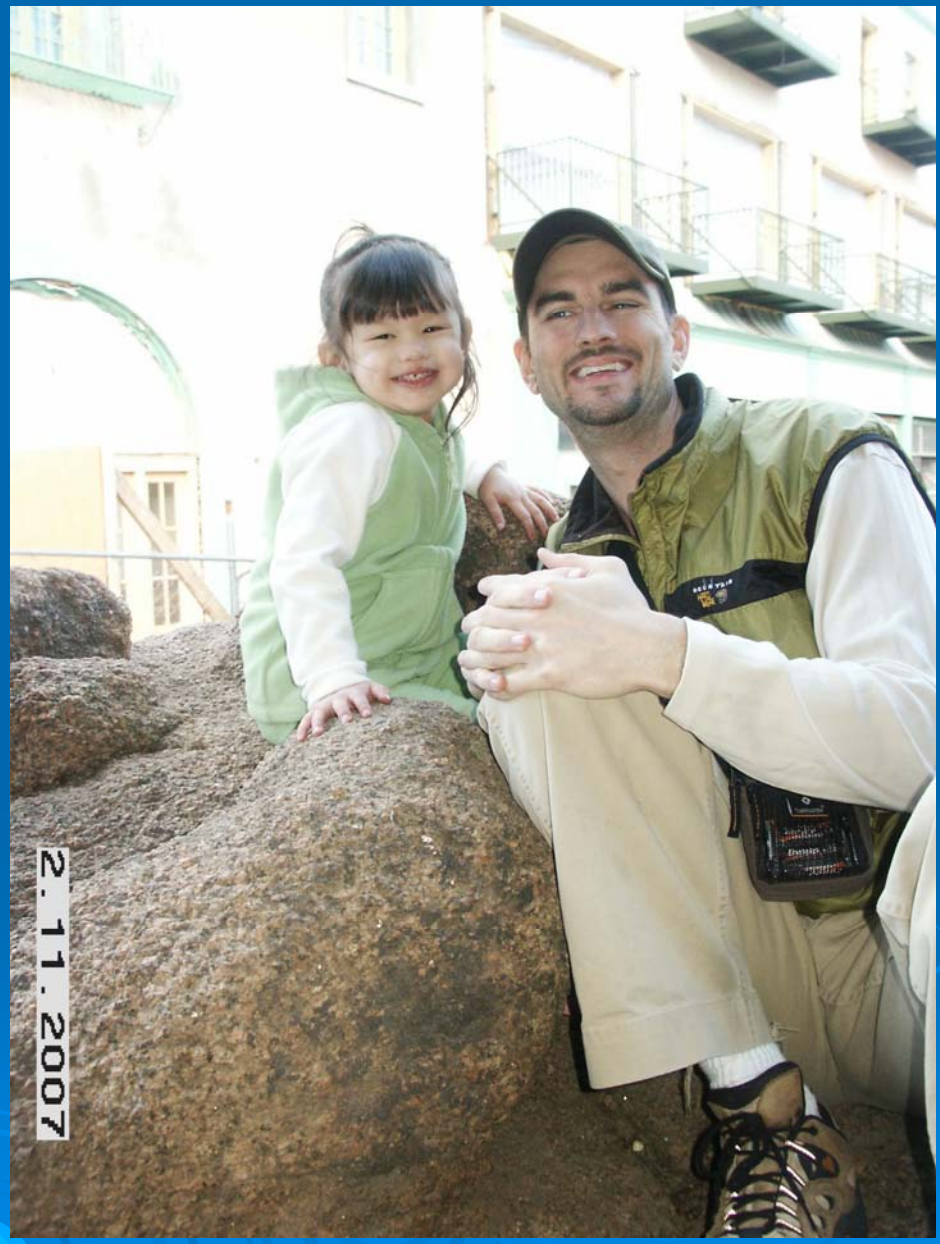
# Acknowledgements


- Philip Portoghese
- Sandy Roerig
- Natalie Lenard
- Ping Law
- Chris Etienne

**Funding:**

**1. Work Supported by NIH project Grant DA1509**
**2. NIDA Postdoctoral Fellowship DA18028 (to N.L.)**


11.24.2005


2.11.2007

# Distance between recognition sites in Mu-Mu opioid receptor models using two TM helices as the interface between dimers

| Dimer Interface | Distance Å |
|---|---|
| TM1,2 – TM2,1 | 41.6 |
| TM1,2 – TM3,2 | 41.0 |
| TM1,2 – TM4,3 | 38.6 |
| TM1,2 – TM5,4 | 39.8 |
| TM1,2 – TM6,5 | 31.8 |
| TM1,2 – TM7,6 | 28.2 |
| TM1,2 – TM1,7 | 38.3 |
| TM2,3 –TM3,2 | 36.7 |
| TM2,3 –TM4,3 | 36.1 |
| TM2,3 –TM5,4 | 38.7 |
| TM2,3 –TM6,5 | 30.0 |
| TM2,3 –TM7,6 | 27.0 |
| TM2,3 –TM1,7 | 38.0 |
| TM3,4 –TM4,3 | 36.4 |

| Dimer Interface | Distance Å |
|---|---|
| TM3,4 –TM5,4 | 37.5 |
| TM3,4 –TM6,5 | 29.5 |
| TM3,4 –TM7,6 | 26.8 |
| TM3,4 –TM1,7 | 29.5 |
| TM4,5 –TM5,4 | 33.8 |
| TM4,5 –TM6,5 | 29.2 |
| TM4,5 –TM7,6 | 30.8 |
| TM4,5 –TM1,7 | 30.2 |
| TM5,6 –TM6,5 | 22.2 |
| TM5,6 –TM7,6 | 19.2 |
| TM5,6 –TM1,7 | 18.7 |
| TM6,7 –TM7,6 | 17.4 |
| TM6,7 –TM1,7 | 24.2 |
| TM7,1 –TM1,7 | 32.4 |

# Antinociceptive activity in the mouse tail-flick assay after acute I.C.V administration

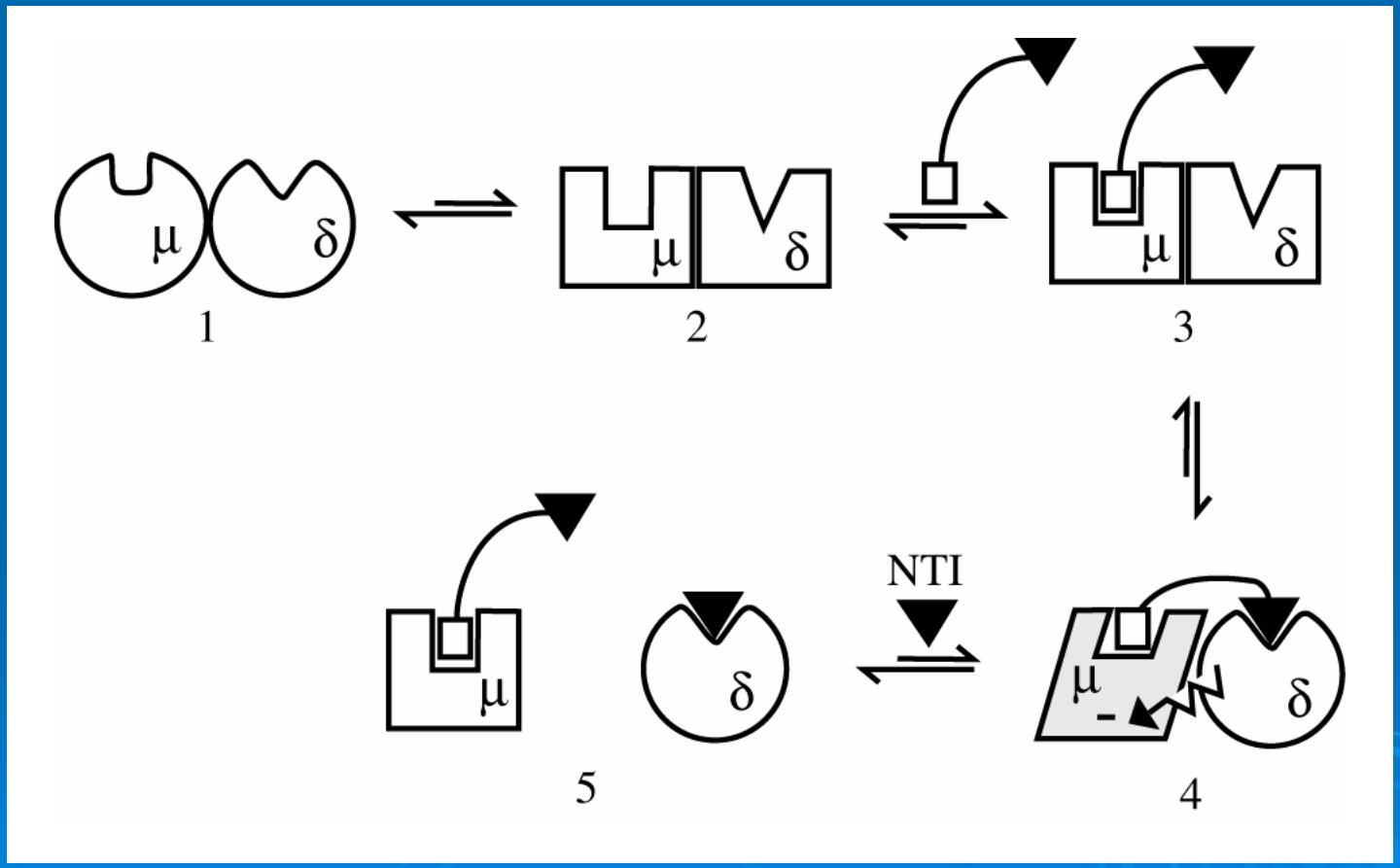
| Ligand | Spacer Length (Å)[a] | $ED_{50}$[b] (95% C.I.) nmol |
|---|---|---|
| **MA-16** | 19.1 | 0.039 (0.032 – 0.046) |
| **MA-17** | 20.4 | 0.040 (0.033 – 0.046) |
| **MA-19** | 22.9 | 0.040 (0.023 – 0.050) |
| **MA-20** | 24.1 | 0.037 (0.029 – 0.045) |
| **MA-21** | 25.4 | 0.044 (0.039 – 0.048) |
| **MDAN-16** | 19.1 | 1.79 (1.54 - 2.04) |
| **MDAN-17** | 20.4 | 1.49 (1.04 - 1.95) |
| **MDAN-18** | 21.6 | 0.95 (0.68 - 1.23) |
| **MDAN-19** | 22.9 | 0.43 (0.36 - 0.50) |
| **MDAN-20** | 24.1 | 0.17 (0.15 - 0.19) |
| **MDAN-21** | 25.4 | 0.08 (0.06 - 0.10) |
| **MA-19 + DN-20** | | 0.037 (0.031 – 0.043) |
| **Oxymorphone** | 25.4 | 0.043 (0.034 – 0.052) |

# NTI Pretreatment

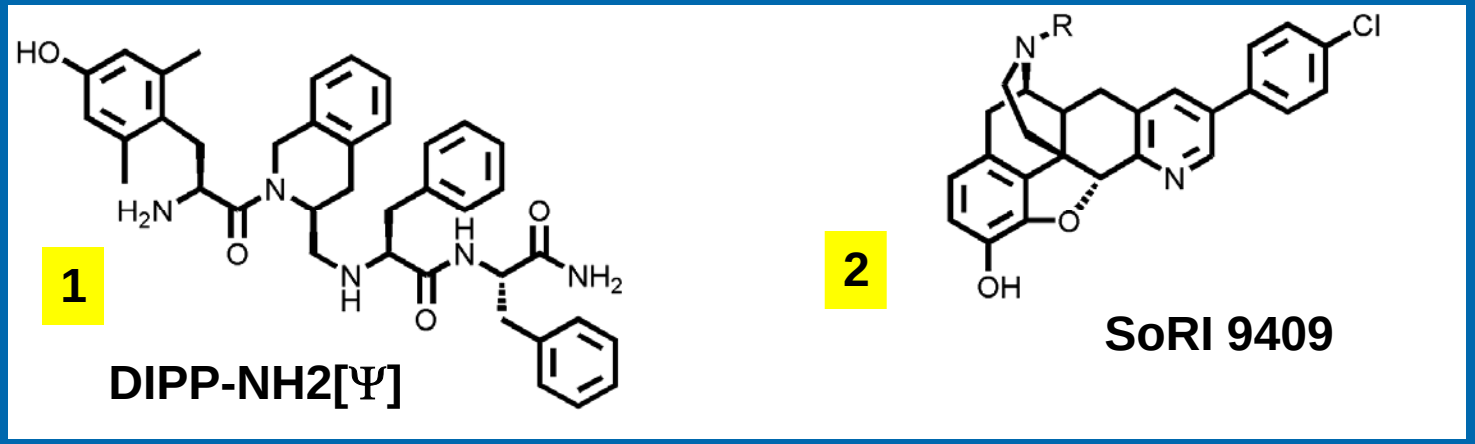
Effect of Pretreatment with Naltrindole on Acute Antinociceptive Potency of Selected μ-δ Bivalent Ligands Administered i.c.v.

| | $ED_{50}$ (95% C.I.) nmol | |
|---|---|---|
| Ligand | No Pretreatment | NTI (50 pmol) Pretreatment |
| **MDAN-16** | 1.70 (1.50 – 2.00) | 0.30 (0.19 – 0.43) |
| **MDAN-19** | 0.43 (0.36 – 0.50) | 0.05 (0.05 – 0.07) |
| **MDAN-21** | 0.08 (0.06 – 0.10) | 0.06 (0.05 – 0.07) |
| **MA-19** | 0.04 (0.023 – 0.045) | 0.08 (0.065 – 0.085) |

# Model for Negative Modulation of Antinociception



# Other Approaches to Avoid Tolerance and Dependence





1 Schiller, P.W.; Fundytus, M.E.; Merovitz, L.; Weltorski, G.; Nguyen, T.M.D.; Lemieux, C.; Chung, N.N.; Coderre, T. The opioid mu agonist/delta antagonist DIPP-NH(2)[Psi] produces a potent analgesic effect, no physical dependence, and less tolerance than morphine in rats. *J. Med. Chem.* **1999**, 42, 3520-3526.

2 Ananthan, S.; Khare, N.K.; Saini, S.K.; Seitz, L.E.; Bartlett, J.L.; Davis, P.; Dersch, C.M.; Porreca, F.; Rothman, R.B.; Bilsky, E.J. Identification of opioid ligands possessing mixed mu agonist/delta antagonist activity among pyridomorphinans derived from naloxone, oxymorphone, and hydromorphone. *J. Med. Chem.* **2004**, 47, 1400-1412