# Opioid Antagonist Activity of Naltrexone-Derived Bivalent Ligands: Importance of a Properly Oriented Molecular Scaffold To Guide "Address" Recognition at κ Opioid Receptors

MariaLaura Bolognesi,[†] William H. Ojala,[§] William B. Gleason,[§] Jane F. Griffin,[▽] Francine Farouz-Grant,[†] Dennis L. Larson,[†] Akira E. Takemori,[‡] and Philip S. Portoghese*,[†]

*Department of Medicinal Chemistry, College of Pharmacy, Department of Pharmacology, Medical School, and Department of Laboratory Medicine and Pathology, Biomedical Engineering Center, Medical School, University of Minnesota, Minneapolis, Minnesota 55455, and Hauptman-Woodward Medical Research Institute, Buffalo, New York 14203*

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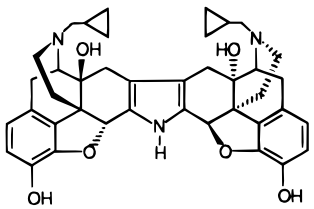
The presence of a molecular scaffold to orient a basic group is important for potent and selective κ opioid antagonist selectivity. An attempt to determine how the geometry of the scaffold affects this selectivity has led to the synthesis of a bivalent ligand (**5**) whose linker constrains the N17′ basic nitrogen (the "address") to a position that is 6.5 Å from N17′ in the κ antagonist norBNI (**1**) when these molecules are superimposed. The fact that compound **5** was found to be a highly selective and potent μ-selective antagonist supports the idea that the position of N17′ in **5** precludes effective ion pairing with the nonconserved residue Glu297 on outer loop 3 of the κ opioid receptor. The high μ receptor binding affinity and *in vitro* pharmacological selectivity of **5** coupled with its presumed low central nervous system bioavailability suggest that it may be a useful antagonist for the investigation of peripheral μ opioid receptors.

## Introduction

The endogenous opioid peptides interact with a family of opioid receptors (μ, δ, κ) which recently have been cloned and sequenced.[1−6] The fact that selective opioid agonists and antagonists[7] have played a prominent role in the pharmacologic characterization and cloning of these receptors has stimulated continued interest in the design of additional selective ligands.

One approach to the the development of highly selective κ opioid antagonists was the bivalent ligand approach,[8] which involved the synthesis of ligands that contain two pharmacophores connected by a linker.[8] This led to the development of norbinaltorphimine (**1**, norBNI), a ligand displaying high selectivity for κ receptors. Norbinaltorphimine is presently widely employed in opioid research.[9]




**1**

Further studies indicated that only one pharmacophore is required for the κ selectivity of norBNI.[10] It was subsequently found that the basic nitrogen (N17′) in the second pharmacophore is essential for κ selectivity, and it was suggested that this basic group functions as an "address" in a manner analogous to Arg[7] of dynorphin.[11,12] In addition, the position of N17′ relative to the antagonist pharmacophore has an influence on the affinity and selectivity for κ receptors.[13] In this report, we describe the results of studies which illustrate the critical nature of the linker that serves as part of a scaffold in orienting the address component in order for it to confer κ antagonist activity and selectivity.

## Chemistry

Initially, one of the principal target compounds in this study was the bivalent ligand **3** which contains two naltrexone-derived pharmacophores whose C-6 centers are part of a spiropiperazine linker. As an approach to the synthesis of **3**, we chose to employ the Strecker reaction[14] on naltrexone (**2**) in order to obtain an α-aminonitrile (**4**) which we planned to employ as an intermediate for **3** (Scheme 1).

When the Strecker synthesis was carried out in an aqueous methanolic solution containing naltrexone (**2**), $NH_4OH$, $NH_4Cl$, and KCN, several products were isolated. These were compound **5** (18.5%), in which the two naltrexone-derived moieties are linked by a 5-amino-4*H*-imidazole, one of the expected aminonitriles **6** (15%), the amide **7** (22%), the amidine **8** (27%), and the hydantoin **10** (3%) (Scheme 1).

The structure of **5** was determined on the basis of spectral data. The mass spectrum of **5** exhibited a molecular ion peak $(M + H)^+$ at 735.4 which is consistent with the molecular formula $C_{42}H_{50}N_6O_6$. However, this also is consistent with diiminopiperazine **3** which is isomeric with **5**. A distinction between **3** and **5** was based on NMR spectral data, inasmuch as only **3** possesses $C_2$ symmetry. Thus, if the product had possessed structure **3**, its naltrexone-derived pharmacophores should be magnetically equivalent. Since the $^1H$ NMR spectrum exhibited a set of proton resonances for each of the pharmacophores, with two clearly separated singlets at 4.49 and 4.69 ppm for the two H-5 protons, this is consistent with the structure of the unsymmetrical dimer **5**. Additional support for structure **5** was obtained by comparing the $^{13}C$ NMR data for the aminoimidazole (C-2 and C-5 at 183 and 190

[†] Department of Medicinal Chemistry.
[‡] Department of Pharmacology.
[§] Department of Laboratory Medicine and Pathology.
[▽] Hauptman-Woodward Medical Research Institute.
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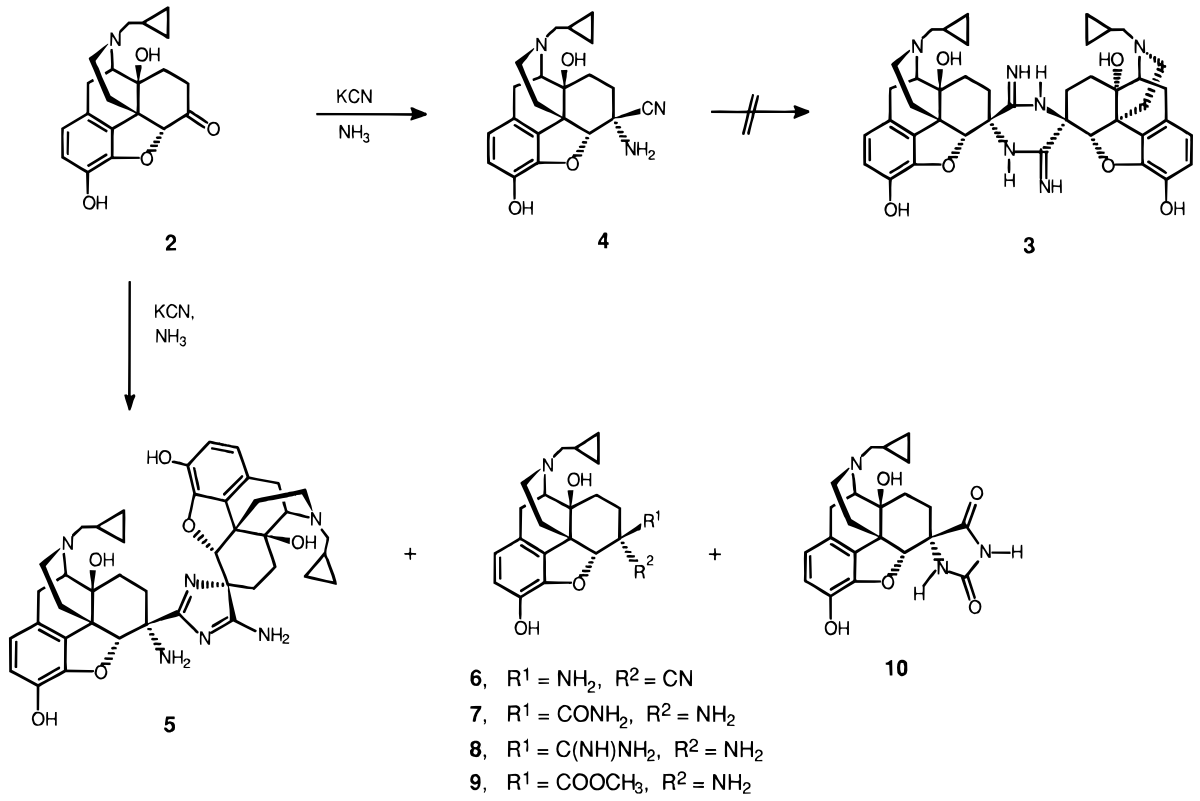
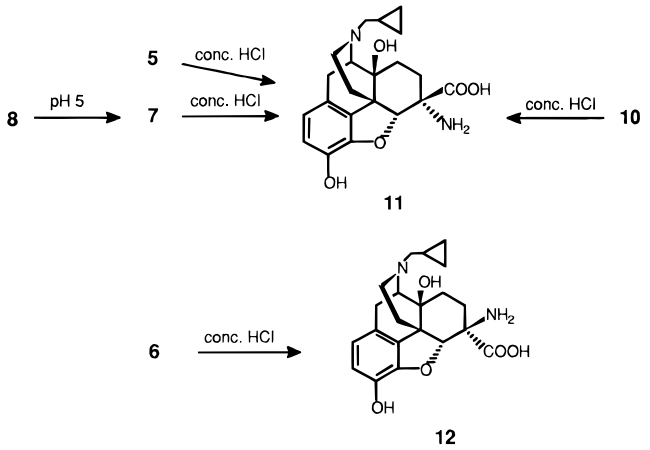
**Scheme 1**




**Scheme 2**



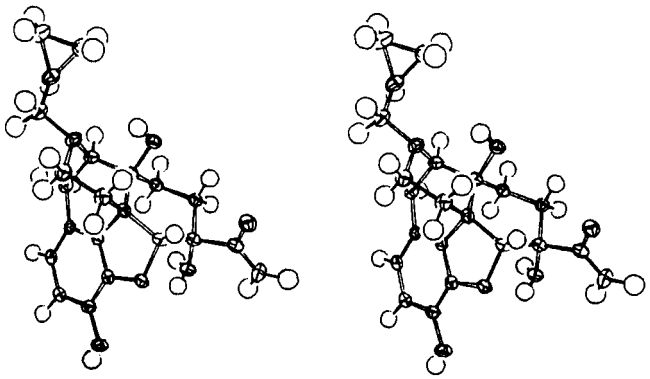



**Figure 1.** X-ray crystal structure of compound **7** represents one of two different structures (A) which differ in the conformation of the cyclopropylmethyl group.

ppm, respectively) with those reported[15] for an analogous system.

Proof for the stereochemistry of compounds **5**, **6**, **8**, and **10** was obtained by chemically relating them to amide **7** (Scheme 2) whose structure (Figure 1) was determined by X-ray crystallography. Hydrolysis of the amidine **8** at pH 5 gave the amide **7** (55%). Hydrolysis of **6** and **7** under strongly acidic conditions yielded the epimeric amino acids **11** and **12**, respectively. Under identical conditions, the hydantoin **10** was hydrolyzed to **11**. Evidence that **5** possesses the same stereochemistry at each of its C-6 centers was provided by subjecting it to strong acid hydrolysis. Only one amino acid (**11**) was produced in 75% yield.

The structure and stereochemistry of **5** were subsequently confirmed by X-ray crystallography (Figure 2). After repeated recrystallizations, a single crystal of **5**·3HCl was obtained from methanol. The overall conformation, and thus the relative orientation of the two pharmacophores, is stabilized by an intramolecular hydrogen bond between the 14-hydroxyl group and imidazole nitrogen as indicated by a close contact of 2.71 Å between the electronegative atoms. This interaction may also contribute to the boat conformation of ring C which is apparent in the 6-aminomorphinan portion of the molecule.

It was found that limited hydrolysis of **5** with dilute aqueous HCl at room temperature afforded imidazolone derivative **13** (45%). Treatment of **5** with acetic anhydride gave the hexaacetate **14** in quantitative yield. Selective acetylation to afford **15** was accomplished using pentafluorophenylacetate.[16]

As there are no reports of ketones affording imidazoles directly in the Strecker reaction, and since only one of the two possible aminonitrile epimers was isolated, we considered a common reactive intermediate to account for the formation of all the products. A reasonable possibility is that the 14-hydroxyl group in **2** facilitates the formation of **5** and **7**−**9** via the reactive

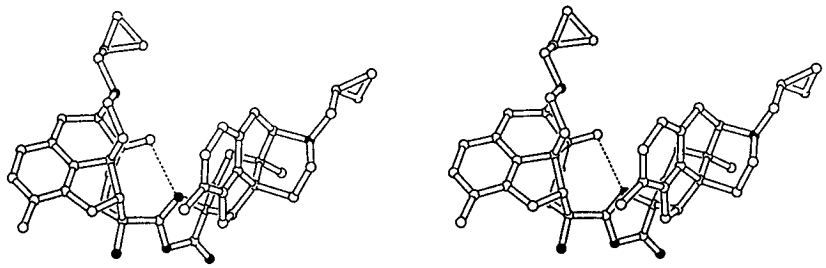
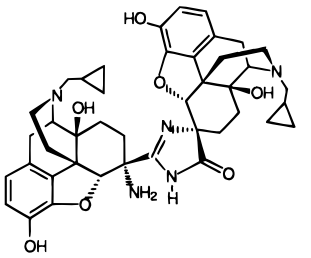
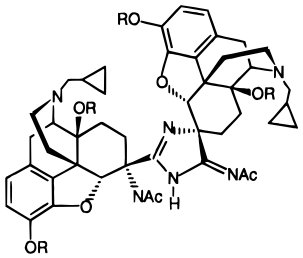


**Figure 2.** X-ray crystal structure of bivalent ligand **5**. Note the putative hydrogen bonding between the 14-hydroxyl group and imidazole nitrogen.



**13**

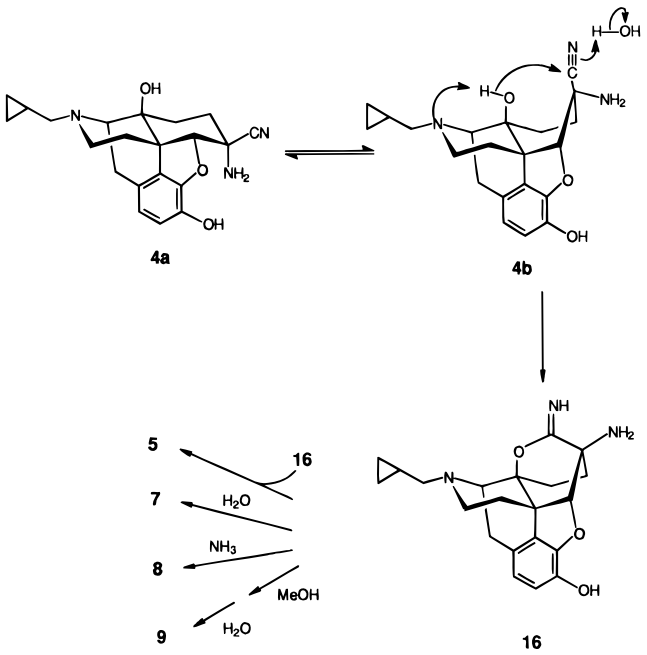


**14**, R = Ac
**15**, R = H

$\delta$-imino lactone intermediate **16** (Scheme 3). This could take place only through one of the epimeric Strecker products **4** because the 14-hydroxyl group can readily attack the nitrile group when ring C is in a boat conformation (**4b**). Facile chair−boat interconversion (**4a** ↔ **4b**) of ring C is reasonable in view of X-ray and NMR evidence that indicate a boat conformation in 6α-substituted opiates.[17,18] The basic nitrogen and 14-hydroxyl group are arranged in a tandem fashion and consequently may facilitate the attack upon the nitrile carbon when ring C is in the boat conformation. Once the $\delta$-imino lactone **16** is formed, its electrophilic imino carbon would readily undergo attack by a variety of nucleophiles in the reaction medium. Thus, nucleophilic attack by the primary amino group of imino lactone **16** upon the imino lactone carbon of another molecule of **16** would lead to an amidine intermediate which would then cyclize to the imidazole **5** via reaction of the amidine nitrogen with the neighboring imino lactone carbon. Apparently, the alternate ring closure leading to the piperazine **3** does not occur to any significant extent because the formation of a 5-membered over a 6-membered ring is entropically favored.

The water and ammonia in the reaction mixture would react with the imino lactone **16** to form the amide **7** and amidine **8**, respectively. The methyl ester **9** was isolated in low yield when the percentage of methanol in the reaction mixture was increased. This could arise via formation of the methyl imidate which would then undergo limited hydrolysis. The formation of the hydantoin **10** may involve the participation of atmospheric $CO_2$ (as $K_2CO_3$) in a reaction similar to the Bucherer synthesis which is used in the preparation of hydantoins from ketones, $(NH_4)_2CO_3$, and KCN.[19]

**Scheme 3**



**4a** **4b** **16**

Finally, it is significant that only the $\beta$-amino-α-nitrile epimer **6** was isolated from the reaction mixture. This is in harmony with the proposed facile neighboring group participation between the 14-hydroxyl and nitrile groups to give the reactive imino lactone intermediate **16**. Apparently, a mixture of epimeric aminonitriles (**4**, **6**) is formed initially, and only one of them (**4**) is then rapidly converted to other products via the imino lactone **16** (Scheme 3). In view of the fact that the total yield of products derived from the proposed reactive intermediate **16** was *ca.* 70%, it is apparent that the amount of its precursor **4** formed in the Strecker reaction was 5-fold greater than that of its epimer **6**. The stereoselective formation of **4** would be consistent with the addition of cyanide ion to the less hindered $\beta$ face of naltrexone if the Strecker reaction proceeds via the Schiff base arising from reaction of ammonia with the ketone group.

## Biological Results

**Smooth Muscle Preparations.** The opioid activity of the synthesized compounds was evaluated on the electrically stimulated guinea pig ileum[20] (GPI) and the mouse vas deferens[21] (MVD) preparations as reported previously.[22] The ligands were incubated with the preparation for 15 min prior to testing. The standard agonists morphine (M), ethylketazocine (EK), and

**Table 1.** Opioid Antagonist Potencies of Naltrexone-Derived Ligands in the GPI and MVD Preparations

| | $IC_{50}$ ratio ± SEM[a,b]/$K_e$, nM | | | $K_e$ selectivity ratio | |
|---|---|---|---|---|---|
| compd | M ($\mu$) | EK ($\kappa$) | DADLE ($\delta$) | $\kappa/\mu$ | $\delta/\mu$ |
| **2** | 98 ± 24/1.0 | 19.3 ± 5.9/5.5 | 10.5 ± 2.3/43 | 5.3 | 42 |
| **5** | 65 ± 15[c]/0.31 | 9.1 ± 2.1/12 | 2.8 ± 0.6/57 | 40 | 184 |
| **6** | 79 ± 15/1.3 | 17.5 ± 1.5/6.0 | 2.5 ± 0.3/67 | 4.7 | 52 |
| **7** | 11.2 ± 2.3/9.8 | 3.3 ± 0.8/44 | 0.89 ± 0.20/*d* | 4.5 | |
| **11** | 4.8 ± 1.1/27 | 1.9 ± 0.7/10 | 1.1 ± 0.2/d | | |
| **12** | 154 ± 35/0.65 | 6.2 ± 1.5/19 | 1.7 ± 0.5/139 | 30 | 214 |
| **13** | 11.8 ± 2.8[f]/0.46 | 1.1 ± 0.1[c]/*e* | 0.87 ± 0.14/*d* | | |
| **15** | 11.7 ± 2.6[c]/1.9 | 3.3 ± 0.5/44 | 1.4 ± 0.5/*d* | 23 | |

[a] The morphine (M) and ethylketazocine (EK) $IC_{50}$ ratios were obtained from the GPI, and the $IC_{50}$ ratios for [D-Ala$^2$,D-Leu]enkephalin (DADLE) were obtained on MVD. [b] Unless otherwise specified, the concentration of the compounds tested was 100 nM. [c] Determined at 20 nM. [d] Not calculated because $IC_{50}$ ratio was not significantly >1. [e] Not determined because of strong agonist activity. [f] Determined at 5 nM.

**Table 2.** Comparison of the Opioid Antagonist Potencies of **5** with Those of Naltrexone (**2**) in the GPI and MVD Preparations

| | | **5** | | **2** | |
|---|---|---|---|---|---|
| agonist[a] | selectivity | $K_e$, nM | $K_e$ ratio[b] | $K_e$, nM | $K_e$ ratio[b] |
| DAMGO | $\mu$ | 0.07 | | 1.2 | |
| EK | $\kappa$ | 12 | 171 | 5.5 | 4.6 |
| U-50488 | $\kappa$ | 4.7 | 67 | 37 | 31 |
| DADLE | $\delta$ | 57 | 814 | 43 | 36 |
| DPDPE | $\delta$ | 66 | 943 | 69 | 58 |

[a] The $K_e$ values were obtained from $IC_{50}$ ratio values ($n \geq 3$) in the GPI and the MVD as described in Table 1. [b] The $K_e$ ratios of **5** or **2**: $\kappa$ agonist/DAMGO or $\delta$ agonist/DAMGO.

**Table 3.** Opioid Receptor Binding of **2**, **5**, and **13** to Guinea Pig Brain Membranes

| | $K_i$, nM[a] | | | $K_i$ ratio | |
|---|---|---|---|---|---|
| | $\mu$[b] | $\delta$[c] | $\kappa$[d] | $\delta/\mu$ | $\kappa/\mu$ |
| **2** | 0.75 | 20 | 36 | 26 | 49 |
| **5** | 0.98 | 183 | 88 | 187 | 90 |
| **13** | 1.24 | 187 | 5.8 | 151 | 5 |

[a] Geometric mean of three replicate determinations. [b] Determined using [$^3$H]DAMGO. [c] Determined using [$^3$H]DADLE + DAMGO (1 $\mu$M). [d] Determined using [$^3$H]EKC + DAMGO (1 $\mu$M).

[D-Ala$^2$,D-Leu$^5$]enkephalin (DADLE) were employed when testing for antagonist activity. They are selective $\mu$, $\kappa$, and $\delta$ opioid agonists, respectively. Three or more replicate determinations were carried out for each compound. The antagonist potency is expressed as an $IC_{50}$ ratio, which is the $IC_{50}$ of the agonist in presence of the antagonist (usually 100 nM) divided by the control $IC_{50}$ in the same preparation, or as a $K_e$ value, where $K_e$ = [antagonist]/($IC_{50}$ ratio − 1).

With the exception of **8** and **10**, which displayed full agonist activity ($IC_{50}$ = 18 ± 4 and 71 ± 28 nM, respectively) in the GPI, the compounds were found to be selective $\mu$ antagonists. At a concentration of 1 $\mu$M, some of the antagonists displayed partial agonist activity, but this did not exceed 21% of a full agonist response.

The compounds exhibited greater antagonism toward morphine than to EK or DADLE (Table 1). The most potent ligand was the aminoimidazole **5**, which was found to be 3 times more potent than naltrexone (**2**) and with a better selectivity profile. A more complete comparison of the antagonist potencies (Table 2) shows that **5** is considerably more $\mu$-selective than naltrexone (**2**). The two monovalent derivatives **6** and **12**, which contain a 6$\beta$-amino group, also possessed good $\mu$ antagonist potency. On the other hand, the amide **7** and carboxylic acid **11**, both of which contain a 6α-amino acid group, exhibited a feeble antagonist activity.

**Binding**. The affinities of **5** and **13** were determined by competition with radioligands for binding sites in guinea pig brain membranes employing a modification of the method of Werling *et al.*[24] Binding to $\mu$ sites was determined using [$^3$H][D-Ala$^2$,MePhe$^4$,Gly-ol$^6$]enkephalin[25] (DAMGO), to $\kappa$ sites with [$^3$H]EK in the presence of 1 $\mu$M DAMGO, and to $\delta$ sites with [$^3$H]-DADLE in the presence of 1 $\mu$M DAMGO. Both bivalent ligands **5** and **13** exhibited $\mu$-selective binding, with **5** having higher selectivity (Table 3). Although **5** possessed approximately the same affinity as naltrexone (**2**) for $\mu$ sites, it exhibited greater selectivity.

***In Vivo* Studies.** The most potent and most $\mu$-selective antagonist (**5**) was tested using the tail-flick procedure in Swiss Webster mice.[26] Administration of **5** (6.2 $\mu$mol/kg sc) and standard agonists was timed so that the peak effect (30 min) coincided with the center of the observation period. The $ED_{50}$ values of the standard agonists [D-Pen$^2$,D-Pen$^5$]enkephalin[27] (DPDPE) ($\delta$), morphine ($\mu$), and *trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide[28] (U-50488) ($\kappa$) were then determined and expressed as $ED_{50}$ ratios (treated/control). The results (Table 4) indicated that morphine was antagonized to the greatest degree ($ED_{50}$ ratio = 4), whereas DPDPE and U-50488 were not significantly antagonized. No agonism for **5** was observed at times ranging from 15 min to 4 h at this dose.

Naltrexone in a sc dose of 50 nmol/kg was found to give a similar morphine $ED_{50}$ ratio of 4.2 (2.9−5.9). The fact that the dose was <1/100th that of **5** suggests access to the central nervous system (CNS) may be responsible for the relatively low antagonist potency of **5** *in vivo*.

## Discussion

Structure−activity relationship studies of bimorphinan ligands related to the prototypical $\kappa$-selective opioid antagonist norBNI (**1**), have revealed that its selectivity is dependent on the geometry of the linker connecting the two naltrexone-derived pharmacophores.[13] It was later found that the second pharmacophore of norBNI was merely serving as part of a scaffold to orient the second basic nitrogen (N17′).[11] The fact that the $\delta$ antagonist naltrindole (**17**), could be converted to a $\kappa$ antagonist (**18**) by a relatively simple molecular modification involving the attachment of a methylamidine group to its indole moiety has corroborated the requirement of a properly oriented basic group.[29] We have proposed that this basic group interacts with a subsite that is unique to the $\kappa$ opioid receptor, possibly an acidic

**Table 4.** Antagonism by **5** of the Antinociceptive Effect of Opioid Agonists in Mice

| | | $ED_{50}$, nmol/mouse or $\mu$mol/kg | | |
|---|---|---|---|---|
| agonist | selectivity | control | treated[a] | $ED_{50}$ ratio[b] |
| morphine[d] | $\mu$ | 17.46 (13.18−22.52) | 68.74 (51.78−88.61) | 4.00 (2.70−5.56) |
| DPDPE[c] | $\delta$ | 24.29 (18.84−31.66) | 16.00 (11.87−21.57) | 0.66 (0.44−0.97 |
| U-50488[d] | $\kappa$ | 35.17 (24.23−47.32) | 25.55 (16.92−36.00) | 0.72 (0.45−1.18) |

[a] Treated sc with 6.2 $\mu$mol/kg **5** (peak time 30 min). [b] Treated $ED_{50}$ divided by control $ED_{50}$. For comparison purposes, the antagonism of morphine by sc naltrexone (50 nmol/kg) gave an $ED_{50}$ ratio of 4.2 (2.9−5.9). [c] Administered icv; values expressed as nmol/mouse. [d] Administered sc $\mu$mol/kg.

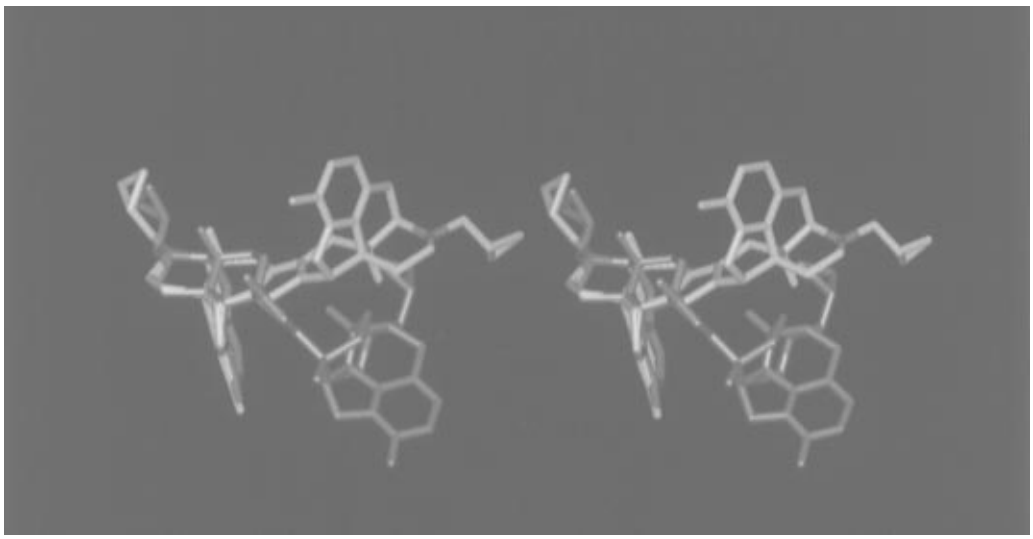
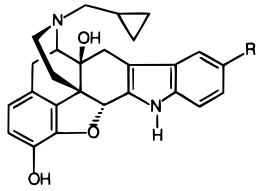


**Figure 3.** Superposition of norBNI (turquoise) upon **5** (magenta). Note the divergent positions of the N17′ basic group.

residue located on one of the extracellular domains.[12] Very recently, the region of the $\kappa$ opioid receptor for this interaction has been localized to extracellular loop 3,[30] and more specifically to Glu297 in this domain.[31]




**17**, R = H
**18**, R = $CH_2NH(C{=}NH)$alkyl

In the present study, the bivalent ligands **5** and **13** exhibited potent $\mu$ opioid antagonist activity in the GPI. In fact, the bivalent ligands were as potent and considerably more selective at $\mu$ receptors than naltrexone. Moreover, the fact that bivalency is not required for potent $\mu$ antagonism indicates that the imidazole linker of **5** and **13** does not significantly contribute to activity.

The dramatic difference in selectivity between norBNI (**1**) and the bivalent ligands in the present series is evidently a reflection of the relative orientation of the naltrexone-derived pharmacophores. This can be seen in the superposition of the X-ray crystal structures of **1**·2HCl and **5**·3HCl (Figure 3). As it appears likely from the X-ray crystal structure that **5**·3HCl is internally hydrogen bonded, the molecule may preferentially assume the same conformation in solution. In this conformation, the N17′ basic nitrogens in morphinan moieties that are not superposed are *ca.* 6.5 Å from one another. This difference between the position of the N17′ in **1** and **5** may be sufficient to prevent the protonated amine in **5** from ion pairing with Glu297 on the $\kappa$ receptor. Thus, high-affinity $\kappa$ antagonists such as **1** and **18** apparently involve a scaffold-directed orientation of the "address" to an acidic residue (Glu297) that is unique to the $\kappa$ receptor.

Finally, this study has led to a potentially valuable $\mu$-selective antagonist (**5**). As **5** possesses only 1% of the central activity exhibited by naltrexone, while having comparable binding affinity and greatly increased selectivity, it may find utility as a pharmacologic tool for the investigation of peripheral[32] $\mu$ opioid receptors.

## Experimental Section

Reagents were purchased from Aldrich Chemicals unless otherwise specified. Naltrexone was supplied by Mallinckrodt. Column chromatography was performed on silica gel (200−400 mesh; Aldrich Chemicals). Spinning thin-layer chromatography was conducted on silica gel from EM Science (silica gel 60, PF254) using a Chromatotron (Harrison Research, Model 7924T). All $R_f$ values were obtained on Analtech analytical silica gel TLC plates. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Chromatographic solvent systems are reported as vol/vol. Infrared spectra were recorded on a Perkin-Elmer 281 or Nicolet 5DXB FTIR instrument. NMR data were collected on a GE Omega 300 MHz NMR instrument at room temperature (18−20 °C). The $\delta$ (ppm) scale was in reference to the deuterated solvent. Mass spectra (high and low resolution) were obtained on a VG707EH-F spectrometer. Elemental analysis was performed by MHW Laboratories, Phoenix, AZ. Optical rotations were determined on a Rudolph Research Autopol III polarimeter.

**Strecker Reaction on Naltrexone (2).** Naltrexone **2** (free base) (6.83 g, 0.02 mol) was dissolved in 1.5:1 MeOH/$H_2O$ (50 mL), after which were added with stirring $NH_4Cl$ (1.28 g, 1.2 equiv), $NH_4OH$ (2.9 g, 1.2 equiv), and KCN (1.5 g, 1.15 equiv). The precipitate that formed after continuous stirring at 23 °C for 6 days was collected, washed successively with water (10

mL) and MeOH (5 mL), and dried *in vacuo*. It was then crystallized from MeOH to yield 1.36 g (18.5%) of **5**: mp >280 °C dec; HPLC $t_R$ 21.7 min (EtOAc/MeOH/$NH_4OH$, 90:10:1, flow rate 3.5 mL/min; Dynamax-60A 21.4 × 250 mm preparative column); $R_f$ 0.30 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 1645, 1609 (C═N); $^1H$ NMR (DMSO-$d_6$) $\delta$ 8.91 (br s, 1H), 8.15−7.49 (m, 2H), 6.43−6.33 (m, 4H), 5.20−4.49 (m, 2H), 4.69 (s, 1H), 4.49 (s, 1H), 3.01−2.88 (m, 4H), 2.61−1.87 (m, 15H), 1.62 (m, 1H), 1.38−0.80 (m, 9H), 0.46−0.43 (m, 4H), 0.08 (m, 4H); $^{13}C$ NMR (DMSO-$d_6$) $\delta$ 190.68, 183.23, 146.60, 145.86, 139.36, 139.17, 133.17, 132.67, 123.81, 123.57, 118.83, 118.51, 117.63, 117.24, 91.46, 90.72, 79.41, 71.04, 70.83, 63.26, 63.19, 59.41, 58.28, 47.49, 46.96, 45.41, 45.29, 32.66, 31.69, 30.59, 29.76, 28.69, 27.49, 23.34, 23.28, 10.31, 4.99, 4.85, 4.67, 4.52; HRFAB-MS calcd for $C_{42}H_{51}N_6O_6$ 735.387 $(M + H)^+$, found 735.388. Anal. ($C_{42}H_{50}N_6O_6 \cdot H_2O$) C, H, N.

The reaction mixture filtrate was evaporated to dryness to give a solid which was purified on a gravity column ($CHCl_3$/MeOH/$NH_4OH$, 9.7:0.5:0.1 to 8:2:0.1) to afford four main fractions. The first high-$R_f$ fraction was identified as the 6$\beta$-amino-6α-nitrile **6** (1.1 g, 15%): mp 185 °C; $R_f$ 0.45 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 2238 (CN); $^1H$ NMR (DMSO-$d_6$) $\delta$ 9.11 (br s, 1H), 6.53 (m, 2H), 4.27 (s, 1H), 3.30−2.96 (m, 4H), 2.62−1.96 (m, 8H), 1.62−1.20 (m, 4H), 0.85−0.75 (m, 1H), 0.48−0.43 (m, 2H), 0.127 (m, 2H); FAB-MS calcd for $C_{21}H_{26}N_3O_3$ 368.197 $(M + H)^+$, found 368.2. Anal. ($C_{21}H_{25}N_3O_3$) C, H, N.

The second fraction consisted of the 6α-amino-6$\beta$-carboxamide **7** (1.7 g, 22%): mp 245 °C; $R_f$ 0.29 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 1681, 1654 (*CO*$NH_2$); $^1H$ NMR (DMSO-$d_6$) $\delta$ 8.99 (br s, 1H), 7.49 (d, $J$ = 1 Hz, 1H), 7.11 (br s, 1H), 6.48 (m, 2H), 5.11 (s, 1H), 4.86 (m, 1H), 4.05 (br s, 2H), 2.95−2.89 (m, 2H), 2.58−1.96 (m, 6H), 1.50−1.14 (m, 6H), 0.81 (m, 1H), 0.43 (d, $J$ = 8.4 Hz, 2H), 0.07 (d, $J$ = 5.1 Hz, 2H); FAB-MS calcd for $C_{21}H_{28}N_3O_4$ 386.208 $(M + H)^+$, found 386.2. Anal. ($C_{21}H_{27}N_3O_4$) C, H, N.

The third consisted of the hydantoin **10** (0.25 g, 3%): mp 198−201 °C; $R_f$ 0.27 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 1723, 1760 (−*CO*NH, HN*CO*NH); $^1H$ NMR ($CD_3OD$) $\delta$ 6.63−6.60 (m, 2H), 4.70 (s, 1H), 3.09−3.03 (m, 2H), 2.65−2.60 (m, 2H), 2.39−2.12 (m, 6H), 1.55 (m, 4H), 1.35−1.30 (m, 4H), 0.86 (m, 1H), 0.52 (m, 2H), 0.13 (m, 2H); FAB-MS calcd for $C_{22}H_{26}N_3O_5$ 412.187 $(M + H)^+$, found 412.1. Anal. ($C_{22}H_{25}N_3O_5$) C, H, N.

The low-$R_f$ fraction was identified as the amidino derivative **8** (2.1 g, 27%); this compound was characterized as the trihydrochloride salt: mp 265 °C dec; $R_f$ 0.04 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 1686 (*C*═*NH*$NH_2$); $^1H$ NMR ($CD_3OD$) 6.76 (m, 2H), 5.05 (s, 1H), 4.87−4.98 (br s, 9H), 4.01 (d, $J$ = 5.4 Hz, 1H), 3.42−3.28 (m, 4H), 3.18−3.10 (m, 2H), 2.94−2.41 (m, 4H), 1.94−1.52 (m, 4H), 1.05−1.20 (m, 1H), 0.83−0.71 (m, 2H), 0.56−0.48 (m, 2H); FAB-MS calcd for $C_{21}H_{29}N_4O_3$ 385.223 $(M + H)^+$, found 385.3. Anal. ($C_{21}H_{31}Cl_3N_4O_3 \cdot 1.5H_2O$) C, H, N, Cl.

When the percentage of MeOH in the solvent mixture MeOH/$H_2O$ was increased to 3:1, the methyl ester **9** was isolated in 3% yield in addition to the abovementioned compounds: mp 110 °C; $R_f$ 0.80 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 1737 (*CO*$OCH_3$); $^1H$ NMR ($CDCl_3$) $\delta$ 6.70 (d, 1H), 6.55 (d, 1H), 5.08 (s, 3H), 3.78 (s, 3H), 3.10−2.98 (m, 3H), 2.66−2.56 (m, 3H), 2.39−2.17 (m, 6H), 1.50−1.25 (m, 4H), 0.83 (m, 1H), 0.55−0.51 (m, 2H), 0.12−0.10 (m, 2H); FAB-MS calcd for $C_{22}H_{29}N_2O_5$ 401.207 $(M + H)^+$, found 401.2. Anal. ($C_{22}H_{28}N_2O_5$) C, H, N.

**Hydrolysis of 8 to 17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-aminomorphinan-6$\beta$-carboxamide (7).** The aminoamidine **8** hydrochloride (20 mg, 0.0421 mmol) was dissolved in $H_2O$ (2 mL), and the solution was stirred at ambient temperature for 3 days. The solvent was evaporated, and the crude material was purified on preparative TLC (silica gel) using $CHCl_3$/MeOH/$NH_4OH$, 95:5:1, as the development solvent to give 8.5 mg (55%) of amide **7** having physical and spectral properties identical with those of **7** obtained from the Strecker reaction.

**Hydrolysis of 7 to 17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-aminomorphinan-6$\beta$-carboxylic Acid (11).** 6α-Amino-6$\beta$-carboxamide **7** (435 mg, 1.13 mmol) was suspended in $H_2O$ (20 mL) and acidified with hydrochloric acid (37%) to pH 0. The resulting solution was heated at 100 °C for 46 h and then evaporated to dryness. The residue was dissolved in MeOH and purified on a preparative TLC column (RP-18 F254S) eluted with MeCN/$H_2O$, 92:8, to yield **11**·2HCl (319 mg, 75%): mp 250 °C; $R_f$ 0.09 ($CHCl_3$/MeOH/$NH_4OH$, 60:40:1); IR (KBr, $cm^{-1}$) 1641, 1629 (*CO*OH); $^1H$ NMR (DMSO-$d_6$) $\delta$ 12.5−11.5 (br s, weak), 7.75−6.75 (br s, 2H), 6.51 (m, 2H), 5.09 (s, 1H), 3.13−2.92 (m, 2H), 2.61−2.01 (m, 8H), 1.58−1.55 (m, 2H), 1.29−1.10 (m, 3H), 0.91−0.82 (m, 1H), 0.45 (d, $J$ = 7.2 Hz, 2H), 0.10 (m, 2H); $[\alpha]^{25}_D$ −49° (MeOH 1%); FAB-MS calcd for $C_{21}H_{27}N_2O_5$ 387.192 $(M + H)^+$, found 387.3. Anal. ($C_{21}H_{28}Cl_2N_2O_5 \cdot 1.25H_2O$) C, H, N.

**Hydrolysis of 10 to 17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-aminomorphinan-6$\beta$-carboxamide (7).** Compound **10** (20 mg, 0.049 mmol) was mixed with concentrated HCL (5 mL) and heated at 100 °C for 5 days. The solution was evaporated to dryness, and the crude mixture was purified by preparative TLC (RP-18 F254S) using MeCN/$H_2O$, 92:8, to furnish 7.5 mg (42%) of **11**: [a]$^{25}_D$ −50°; FAB-MS found 387 $(M + H)^+$.

**Hydrolysis of 6 to 17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6$\beta$-aminomorphinan-6α-carboxylic Acid (12).** A solution of 6$\beta$-amino-6α-nitrile **6** (45 mg, 0.12 mmol) in 3 mL of hydrochloric acid (37%) was heated to 100 °C for 48 h and then evaporated to dryness. The residue was dissolved in MeOH and purified on a preparative TLC column (RP-18 F254S) eluted with MeCN/$H_2O$ (8:2) to yield **12**·2HCl (38 mg, 69% yield): mp 260 °C dec; $R_f$ 0.11 ($CHCl_3$/MeOH/$NH_4OH$, 60:40:1); $^1H$ NMR ($CD_3OD$) $\delta$ 6.66−6.56 (m, 2H), 4.90 (br s, 2H), 4.48 (s, 1H), 3.68 (d, $J$ = 7.2 Hz, 2H), 3.29−3.18 (m, 2H), 2.96−2.80 (m, 3H), 2.75−2.43 (m, 3H), 1.75−1.58 (m, 6H), 1.26 (m, 1H), 1.01−0.97 (m, 1H), 0.45−0.62 (m, 2H), 0.36−0.33 (m, 2H); FAB-MS calcd for $C_{21}H_{27}N_2O_5$ 387.192 $(M + H)^+$, found 387.3. $[\alpha]^{25}_D$ −118.5° (MeOH, 1%). Anal. ($C_{21}H_{28}Cl_2N_2O_5 \cdot H_2O$) C, H, N, Cl.

**Hydrolysis of 5 to 17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-aminomorphinan-6$\beta$-carboxylic Acid (11).** A solution of **5** (30 mg, 0.041 mmol) in 3 mL of hydrochloric acid (37%) was heated to 100 °C for 24 h. The reaction mixture was then evaporated to dryness, and the crude compound was purified on a preparative TLC column (RP-18 F254S) eluted with MeCN/$H_2O$ (92:8) to yield **11**·2HCl (27 mg 75%): mp 251 °C; $[\alpha]^{25}_D$ −50° (MeOH, 1%).

**Hydrolysis of 5 to 2′-[17-(Cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-amino-6$\beta$-morphinanyl]-17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxyspiro[morphinan-6,4′-imidazolin]-5′-one (13).** Concentrated HCl (0.2 mL) was added to a mixture of 100 mg of **5** (0.136 mmol) in 2 mL of water. The resulting solution was stirred at room temperature for 60 h. The solvent was evaporated under vacuum at 50 °C, and the crude material was purified with gravity column chromatography ($CHCl_3$/MeOH/$NH_4OH$, 9.5:0.5:0.1) to afford 45 mg of **13** (45% yield). The recovery of **5** amounted to 55 mg (55%): mp >280 °C dec; $R_f$ 0.35 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 1625, 1641, 1728, 1738; $^1H$ NMR (DMSO-$d_6$) $\delta$ 10.7−10.2 (br s, weak, 2H), 8.9−8.4 (br s, weak, 1H), 6.49−6.42 (m, 4H), 4.95−4.91 (m, 2H), 4.61 (s, 1H), 4.45 (s, 1H), 2.99−2.86 (m, 4H), 2.61−1.95 (m, 10H), 1.72 (t, 2H), 1.53 (t, 2H), 1.31−0.80 (m, 8H), 0.88−0.80 (m, 2H), 0.45−0.43 (m, 4H), 0.08 (m, 4H); FAB-MS calcd for $C_{42}H_{50}N_5O_7$ 736.371 $(M + H)^+$, found 736.4. Anal. ($C_{42}H_{49}N_5O_7 \cdot 1.5H_2O$) C, H, N.

**Acetylation of 5 to 5′-(Acetylimino)-2′-[17-(cyclopropylmethyl)-4,5α-epoxy-3,14-diacetoxy-6α-(acetylamino)-6$\beta$-morphinanyl]-17-(cyclopropylmethyl)-4,5α-epoxy-3,-14-diacetoxyspiro[morphinan-6,4′-4′*H*-imidazole] (14).** A solution of **5** (25 mg, 0.034 mmol) in acetic anhydride (5 mL) was refluxed for 1 h and then evaporated to dryness. The residue was dissolved in chloroform (10 mL) and washed with $NaHCO_3$-saturated solution (2 × 5 mL) and $H_2O$ (2 × 5 mL). The organic layer was then dried over $Na_2SO_4$ and evaporated to dryness to afford 30 mg (90%) of **14**: $^1H$ NMR ($CDCl_3$) $\delta$ 11.01 (br s, weak), 6.90−6.66 (m, 4H), 6.28 (s, 1H), 5.02 (s, 1H), 4.73 (s, 1H), 4.39 (t, 2H), 3.14−3.08 (m, 2H), 2.80−

1.94 (m, 36H), 1.72−1.20 (m, 8H), 0.78−0.74 (m, 2H), 0.49−0.47 (m, 4H) 0.06 (m, 4H); IR (KBr, $cm^{-1}$) 1760, 1734, 1680, 1648; FAB-MS calcd for $C_{54}H_{63}N_6O_{12}$ 987.450 $(M + H)^+$, found 987.6. Anal. ($C_{54}H_{62}N_6O_{12}$) C, H, N.

**Acetylation of 5 to 5′-(Acetylimino)-2′-[17-(cyclopropylmethyl)-4,5α-epoxy-3,14-dihydroxy-6α-(acetylamino)-6β-morphinanyl]-17-(cyclopropylmethyl)-4,5α-epoxy-3,-14-dihydroxyspiro[morphinan-6,4′-4′*H*-imidazole] (15).** Pentafluorophenylacetate (51 mg, 0.225 mmol) was added to a solution of **5** (55 mg, 0.075 mmol) in dry DMF (0.5 mL). The reaction mixture was stirred at room temperature for 6 h, and the solvent was removed *in vacuo*. The crude material was purified using spinning thin-layer chromatography (1-mm plate; $CHCl_3$/MeOH/$NH_4OH$, 98:2:1) to give 9 mg of **15** (15%): mp >280 °C dec; $R_f$ 0.61 ($CHCl_3$/MeOH/$NH_4OH$, 90:10:1); IR (KBr, $cm^{-1}$) 1580, 1659; $^1H$ NMR ($CDCl_3$) $\delta$ 11.4 (br s, weak, 2H), 6.85−6.56 (m, 4H), 5.11 (s, 1H), 4.84 (s, 1H), 3.17−2.88 (m, 4H), 2.62−2.74 (m, 4H), 2.50 (s, 3H), 2.44−2.32 (m, 8H), 2.30 (s, 3H), 2.21−1.85 (m, 6H), 1.55−1.11 (m, 8H), 0.88−0.80 (m, 2H), 0.59−0.51 (m, 4H), 0.15−0.12 (m, 4H); FAB-MS calcd for $C_{46}H_{55}N_6O_8$ 819.408 $(M + H)^+$, found 819.5. The purity of **15** was further evaluated in a different solvent system: $R_f$ 0.21 (EtOAc/MeOH/$NH_4OH$, 85:15:1).

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**Supporting Information Available:** Experimental conditions for data collection in the X-ray crystallography and the positional parameters of compounds **5** and **7** (17 pages). Ordering information can be found on any current masthead page.

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