Synthesis and in Vitro and in Vivo Activity of (−)-(1*R*,5*R*,9*R*)- and (+)-(1*S*,5*S*,9*S*)-*N*-Alkenyl-, -*N*-Alkynyl-, and -*N*-Cyanoalkyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan Homologues

Everette L. May,*,† Arthur E. Jacobson,‡ Mariena V. Mattson,‡ John R. Traynor,§ James H. Woods,§ Louis S. Harris,† Edward R. Bowman,† and Mario D. Aceto†

Department of Pharmacology and Toxicology, Virginia Commonwealth University, Richmond, Virginia 23298-0613, Laboratory of Medicinal Chemistry, Building 8, Room B1-22, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892-0815, and Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109

Received July 25, 2000

Two of the synthesized (−)-(1*R*,5*R*,9*R*)-*N*-homologues (*N*-but-3-enyl- and *N*-but-3-ynyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan (**9**, **13**)) were found to be about 20 times more potent than morphine in the mouse tail-flick assay (ED_{50} = 0.05 mg/kg), and (−)-(1*R*,5*R*,9*R*)-*N*-but-2-ynyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan ((−)-(1*R*,5*R*,9*R*)-*N*-but-2-ynylnormetazocine, **12**) was about as potent as the opioid antagonist *N*-allylnormetazocine (AD_{50} in the tail-flick vs morphine assay = 0.3 mg/kg). All of the homologues examined had higher affinity for the κ-opioid receptor than the μ-receptor except (−)-*N*-but-2-ynyl-normetazocine (**12**), which had a κ/μ ratio = 7.8 and a δ/μ ratio = 118. The (−)-*N*-2-cyanoethyl (**3**), -allyl (**8**), and -but-3-ynyl (**13**) analogues had good affinity (<10 nM) for δ-opioid receptors. Two homologues in the (+)-(1*S*,5*S*,9*S*)-normetazocine series, *N*-pent-4-enyl (**24**) and *N*-hex-5-enyl (**25**), were high-affinity and selective σ_1-ligands (K_i = 2 nM, σ_2/σ_1 = 1250, and 1 nM, σ_2/σ_1 = 750, respectively); in contrast, *N*-allylnormetazocine (**22**) had relatively poor affinity at σ_1, and its σ_1/σ_2 ratio was <100.

Introduction

The influence of some *N*-substituents in the 6,7-benzomorphan, morphinan, and 4,5-epoxymorphinan families of opioids on in vivo activity is well-known when these compounds exert their effects primarily through the μ-opioid receptor. Far less is known about the effect of these *N*-substituents on ligands that selectively interact with δ- or κ-opioid receptors. Thus, (−)-(1*R*,5*R*,9*R*)-*N*-methyl- and *N*-pentyl-substituted 5,9-dimethyl-2′-hydroxy-6,7-benzomorphan ((−)-(1*R*,5*R*,9*R*)-metazocine and (−)-(1*R*,5*R*,9*R*)-*N*-pentylnormetazocine, respectively) have both been found to be potent antinociceptives with little narcotic antagonist activity, and *N*-propyl- and *N*-allyl-substituted normetazocines display potent narcotic antagonist actions, with little, if any, agonist activity.[1] These *N*-substituents exert similar in vivo effects in the morphinan and 4,5-epoxymorphinan families. Data such as these enable a priori prediction that *N*-substituents bearing three-carbon chains in the morphinan and benzomorphan types of opioids will display opioid antagonist actions in vivo and that *N*-methyl and *N*-pentyl substituents confer potent antinociceptive activity in these types of compounds, presumably through μ-opioid receptors. However, the effects of other alkenyl *N*-substituents, *N*-alkynyl, or *N*-cyanoalkyl chains on an opioid ligand are lesser known and have not been systematically studied. Molecular modeling and docking studies of opioids,[2−5] which theoretically could qualitatively indicate ligand activity, are inconsistent thus far and do not appear as yet to offer much guidance for the prediction of in vivo activity or in vitro binding of opioids bearing novel *N*-substituents. We have, thus, undertaken the synthesis and pharmacological evaluation of a number of *N*-substituted *N*-normetazocines since there is, to our knowledge, no way of even qualitatively predicting the varied narcotic agonist or antagonist activities conferred by new *N*-substituents on opioids.

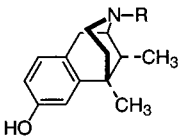
It was previously found that racemic[6,7] and (−)-*N*-2-cyanoethyl-*N*-normetazocines were very potent antinociceptive agents.[8] (±)-*N*-Cyanomethyl-*N*-normetazocine was found to be considerably less potent in vivo than the (±)-*N*-2-cyanoethyl-*N*-normetazocine and had only one-seventh its affinity for the heterogeneous opioid receptor mixture used in binding affinity experiments at that time. (±)-*N*-3-Cyanopropyl-*N*-normetazocine, although 10-fold less antinociceptively potent in the mouse hot-plate assay, was found to have essentially the same binding affinity as the (±)-*N*-2-cyanoethyl compound.[8] The effect of an *N*-cyanoalkyl substituent on the activity of *N*-normetazocine was replicated in the morphinan series of analgesics but, surprisingly, not in the 4,5-epoxymorphinans studied.[8] The (−)-*N*-2-cyanoethyl-4,5-epoxymorphinans which were examined were either inactive or much less potent as antinociceptives.

To systematically examine the effect of chain length of an *N*-substituent bearing an unsaturated moiety, as well as to confirm and extend previous findings with the cyanoalkyl moiety, we prepared an extensive series (Table 1) of enantiomeric *N*-substituted normetazocines,

* To whom correspondence should be addressed. Tel: 804-828-2064. Fax: 804-786-1532. E-mail: ljohnson@hsc.vcu.edu.
† Virginia Commonwealth University.
‡ National Institute of Diabetes and Digestive and Kidney Diseases.
§ University of Michigan Medical School.

10.1021/jm000317+ CCC: $19.00
Published on Web 12/02/2000

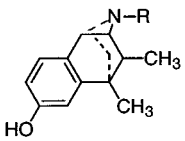
Table 1. Structures, Melting Points, and Optical Rotations[a] of Enantiomeric *N*-Alkenyl-, *N*-Alkynyl-, and *N*-Cyanoalkylbenzomorphan Homologues



(-)-(1*R*,5*R*,9*R*) Series

1: R=H

8: R=$CH_2CH{=}CH_2$



(+)-(1*S*,5*S*,9*S*) Series

15: R=H

22: R=$CH_2CH{=}CH_2$

R	no.	$[\alpha]^{22}_D$	concn	mp, °C	R	no.	$[\alpha]^{22}_D$	concn	mp, °C
CH_2CN	**2**	−91.7	0.288	200−203	CH_2CN	**16**	+90.5	0.243	200−203
$(CH_2)_2CN$	**3**	−88.8	0.224	222−223	$(CH_2)_2CN$	**17**	+91.1	0.202	222−223
$(CH_2)_3CN$	**4**[b]	−92.7	0.206	127−128	$(CH_2)_3CN$	**18**[b]	+94.8	0.192	127−128
$(CH_2)_4CN$	**5**	−72.4	0.225	100 and 204	$(CH_2)_4CN$	**19**	+72.7	0.183	100 and 204
$(CH_2)_5CN$	**6**	−77.7	0.153	203−204	$(CH_2)_5CN$	**20**	+77.9	0.172	203−204
$(CH_2)_6CN$	**7**	−74.9	0.283	172−173	$(CH_2)_6CN$	**21**	+75.6	0.242	172−173
$(CH_2)_2CH{=}CH_2$	**9**	−73.2	0.272	185−186	$(CH_2)_2CH{=}CH_2$	**23**	+73.2	0.340	185−187
$(CH_2)_3CH{=}CH_2$	**10**	−75.2	0.318	100	$(CH_2)_3CH{=}CH_2$	**24**[b]	+74.9	0.283	95
$(CH_2)_4CH{=}CH_2$	**11**	−80.1	0.195	225−227	$(CH_2)_4CH{=}CH_2$	**25**	+79.7	0.295	225−227
$CH_2C{\equiv}CCH_3$	**12**	−102	0.252	233	$CH_2C{\equiv}CCH_3$	**26**	+106	0.178	233
$(CH_2)_2C{\equiv}CH$	**13**	−93.6	0.261	205−207	$(CH_2)_2C{\equiv}CH$	**27**	+91.0	0.203	207−208
$(CH_2)_3C{\equiv}CH$	**14**[b]	−91.5	0.158	135−138	$(CH_2)_3C{\equiv}CH$	**28**[b]	+92.6	0.480	137−139

[a] Optical rotations were determined in MeOH; concentration is in g/100 mL. [b] Free base (all others are HCl salts).

including *N*-cyanoalkyl-*N*-normetazocines (*N*-cyanomethyl to *N*-6-cyanohexyl, **2**−**7**), *N*-alkenyl-*N*-normetazocines (*N*-allyl to *N*-hex-5-enyl, **8**−**11**), and *N*-alkynyl analogues (*N*-but-2-ynyl, *N*-but-3-ynyl, and *N*-pent-4-ynyl, **12**−**14**), and determined their effects in vivo (antinociceptive activity in the mouse tail-flick, phenylquinone, and hot-plate assays and single-dose suppression of morphine abstinence in the monkey) and in vitro (μ-, κ-, δ-opioid and σ_1- and σ_2-receptor binding assays). The data were perused in the perspective of our previous exploration of the *N*-alkyl-substituted normetazocines.[1] The preparation of higher homologues of an *N*-allyl moiety necessitated the determination of their interaction with σ-binding sites (σ_1 and σ_2), for comparison with (±)-*N*-allylnormetazocine (SKF 10,047) which had been considered the prototypic σ-ligand,[9,10] for an opioid receptor, before the σ-site was rejected as an opioid receptor subtype.[11,12] Both racemic and (+)-*N*-allylnormetazocine have also been found to induce phencyclidine-like behavioral effects in monkeys and rats.[13] Many (+)-(1*S*,5*S*,9*S*)-*N*-substituted normetazocines are known to interact with high affinity to σ-sites.[1,14,15] (+)-*N*-Allylnormetazocine (**22**) does not have opioid-like actions in vivo, and the (−)-*N*-allyl enantiomer **8**, a fairly potent opioid antagonist, has some, but much less affinity than its (+)-enantiomer for the σ-site. We have previously examined the interaction of ligands with σ-sites and summarized the role they are said to play in a number of biological functions.[1,14,16] Last, we examined the effect of several *N*-alkynyl substituents in normetazocine, variants of the known racemic,[17] (−)-, and (+)-*N*-propynylnormetazocines. (−)-*N*-Propynylnormetazocine has been found to be a weak opioid antagonist; its estimated potency was about 0.04 that of naloxone in the precipitated withdrawal assay in monkeys; antinociceptive activity was found only in the mouse phenylquinone assay, not in the hot-plate or tail-flick assays.[18,19] Consequently, it was instructive to prepare and examine the activities of a methyl-substituted propynyl substituent (*N*-but-2-ynyl, **12**), as well as the but-3-ynyl (**13**) and pent-4-ynyl (**14**) analogues.

Chemistry

Compounds in Table 1 were prepared from (−)-(1*R*,5*R*,9*R*)- and (+)-(1*S*,5*S*,9*S*)-*N*-normetazocines (**1** and **15**, respectively, R = H) and ω-bromoalkyl cyanides, alkenes, or alkynes in refluxing THF−DMF (4:1) with $KHCO_3$ as the HBr acceptor, reaction time 1−8 h. The course of reaction was monitored by TLC (silica gel GHLF, Analtech Uniplates; solvent system 90:9:1 CH_2Cl_2−MeOH−NH_4OH with I_2 and/or UV detection). Yields were 70−90%. Melting points and optical rotations (HCl salts except **3** and **13**, free bases) are given in Table 1.

Results

In the (−)-(1*R*,5*R*,9*R*)-*N*-cyanoalkyl series, the 2-cyanoethyl (**3**), and 3-cyanopropyl (**4**) analogues showed high affinity for the μ-opioid receptor, subnanomolar affinity for the κ-receptor, and moderate affinity for the δ-receptor (Table 2). Analogues with longer (**5** and **6**) or shorter (**2**) cyanoalkyl chains had considerably less affinity for all of the opioid receptors. With only one exception (the (−)-but-2-ynyl analogue **12**), all of the other (−)-(1*R*,5*R*,9*R*)-compounds, no matter the *N*-substituent, displayed higher affinity for the κ-opioid receptor than they did for the μ-opioid receptor. Many 6,7-benzomorphans are known to interact with the κ-opioid receptor with high affinity. The prototypic κ-opioid receptor ligand was considered to be ketocyclazocine,[9,10] a 6,7-benzomorphan from which the κ-receptor derived its name. This occurred prior to the isolation of the opioid receptor types and before identification of U50,488 ((±)-*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)cyclohex-1-yl]benzeneacetamide), its enantiomer,[20] and the dynorphins as very high-affinity, selective κ-ligands.

Table 2. Displacement Studies[a] of (−)-(1*R*,5*R*,9*R*)-Homologues

no.	R (−)	μ	δ	κ	σ_1	σ_2
2	CH_2CN	100 ± 24	590 ± 70	70 ± 10	13000 ± 2000	19000 ± 2000
3	$(CH_2)_2CN$	4.2 ± 0.8	9.8 ± 0.3	0.4 ± 0.1	9800 ± 4000	51000 ± 8000
4	$(CH_2)_3CN$	5.8 ± 0.4	23 ± 4	0.4 ±0.1	1700 ± 300	8400 ± 900
5	$(CH_2)_4CN$	18 ± 0.4	160 ± 40	6.9 ± 0.7	490 ± 45	2800 ± 300
6	$(CH_2)_5CN$	15 ± 2.7	220 ± 40	11 ± 1	640 ± 230	380 ± 50
7	$(CH_2)_6CN$	90 ± 10	350 ± 70	38 ± 7	80 ± 20	150 ± 5
8	$CH_2CH{=}CH_2$	0.9 ± 0.1	5.5 ± 0.4	0.3 ± 0.03	11000 ± 1100	3200 ± 120
9	$(CH_2)_2CH{=}CH_2$	2.1 ± 0.8	21 ± 6	1.3 ± 0.2	1600 ± 160	620 ± 20
10	$(CH_2)_3CH{=}CH_2$	15 ± 7	60 ± 20	5.9 ± 2	50 ± 0.2	180 ± 14
11	$(CH_2)_4CH{=}CH_2$	10 ± 3	56 ± 21	5.8 ± 0.5	22 ± 2	80 ± 10
12	$CH_2C{\equiv}CCH_3$	0.5 ± 0.3	60 ± 10	3.9 ± 0.5	9800 ± 600	4400 ± 200
13	$(CH_2)_2C{\equiv}CH$	4.6 ± 0.5	10 ± 1.6	1.4 ± 0.1	5900 ± 200	2200 ± 250
14	$(CH_2)_3C{\equiv}CH$	18 ± 4	140 ± 30	11 ± 2	200 ± 20	440 ± 25

[a] K_i, nM. All values are mean ± SEM of at least three experiments. [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69,593 at their K_d concentration (0.57, 2.1, and 0.95 nM, respectively) were used for the μ-, δ-, and κ-subtype binding assays, respectively. The K_d concentration of (+)-[^{3}H]pentazocine (2 nM) was used for the σ_1 assay, and [^{3}H]DTG (5 nM), with 1 μM dextrallorphan to mask σ_1 binding, was used for the σ_2 assay.

Table 3. Displacement Studies[a] of (+)-(1*S*,5*S*,9*S*)-Homologues

no.	R (+)	μ	δ	κ	σ_1	σ_2
16	CH_2CN	18.6%	3.0%	20.4%	11000 ± 400	93000 ± 4000
17	$(CH_2)_2CN$	11.6%	5.6%	600 ± 200	250 ± 90	>100000
18	$(CH_2)_3CN$	9.0%	7.5%	240 ± 4	20 ± 5	34000 ± 500
19	$(CH_2)_4CN$	4800 ± 600	10%	1300 ± 40	36 ± 6	13000 ± 2400
20	$(CH_2)_5CN$	12.4%	11.5%	500 ± 40	33 ± 5	5700 ± 540
21	$(CH_2)_6CN$	37%	4%	17.7%	16 ± 3	1900 ± 340
22	$CH_2CH{=}CH_2$	28.5%	2.5%	31%	300 ± 50	27000 ± 2500
23	$(CH_2)_2CH{=}CH_2$	41%	1%	35.5%	15 ± 2	7600 ± 500
24	$(CH_2)_3CH{=}CH_2$	20.5%	0.8%	400 ± 20	2.1 ± 0.3	2500 ± 400
25	$(CH_2)_4CH{=}CH_2$	980 ± 170	4.2%	420 ± 50	1.1 ± 0.1	760 ± 140
26	$CH_2C{\equiv}CCH_3$	not tested	not tested	not tested	160 ± 5	12000 ± 600
27	$(CH_2)_2C{\equiv}CH$	>10000	>10000	1700 ± 400	110 ± 5	15000 ± 1300
28	$(CH_2)_3C{\equiv}CH$	3600 ± 700	>10000	330 ± 40	18 ± 1	3500 ± 850

[a] K_i, nM, or % inhibition at 1 μM. All values are mean ± SEM of at least three experiments except when a percentage value is given, then $n = 2$. [^{3}H]DAMGO, [^{3}H]DPDPE, and [^{3}H]U69,593 at their K_d concentration (0.57, 2.1, and 0.95 nM, respectively) were used for the μ-, δ-, and κ-subtype binding assays, respectively. The K_d concentration of (+)-[^{3}H]pentazocine (2 nM) was used for the σ_1 assay, and [^{3}H]DTG (5 nM), with 1 μM dextrallorphan to mask σ_1 binding, was used for the σ_2 assay.

Table 4. In Vivo Activities of (−)-(1*R*,5*R*,9*R*)-Homologues of *N*-Cyano-, *N*-Alkenyl-, and *N*-Alkynyl-Substituted Benzomorphans

	antinociceptive and opioid antagonist assays (sc, mg/kg)[a]				
R (−) (no.)	TF[b]	PPQ[c]	HP[d]	TF vs M[e]	SDS[f]
CH_2CN (**2**)	12 (5−28)	1.5 (0.5−4.7)	9 (3−23)	inactive[g]	substituted completely
$(CH_2)_2CN$ (**3**)	0.3 (0.01−0.8)	0.05 (0.03−0.1)	0.1 (0.05−0.3)	inactive	partial substitution[h]
$(CH_2)_3CN$ (**4**)	0.4 (0.2−0.7)	0.01 (0.05−0.3)	1 (0.4−3.4)	inactive	partial substitution[h]
$(CH_2)_4CN$ (**5**)	15 (5−59)	3 (1−9)	inactive	inactive	substituted completely
$(CH_2)_5CN$ (**6**)	inactive	inactive	inactive	10 (5−19)	not tested
$(CH_2)_6CN$ (**7**)	inactive	9 (3−27)	inactive	inactive	briefly substituted
$CH_2CH{=}CH_2$ (**8**)	inactive[i]	0.1 (0.05−0.3)[i]	inactive[i]	0.2 (0.1−0.6)[j]	opioid antagonist[j]
$(CH_2)_2CH{=}CH_2$ (**9**)	0.05 (0.01−1.5)	0.3 (0.1−0.7)	0.8 (0.3−2.2)	4 (1−12)	substituted completely
$(CH2)_3CH{=}CH_2$ (**10**)	inactive	1.4 (0.6−3.5)	inactive	6 (2−17)	no substitution
$(CH_2)_4CH{=}CH_2$ (**11**)	0.3 (0.1−0.6)	0.8 (0.4−1.5)	0.6 (0.1−2.2)	Inactive	substituted completely
$CH_2C{\equiv}CCH_3$ (**12**)	inactive	0.1 (0.04−0.3)	inactive	0.3 (0.1−0.7)	exacerbated withdrawal
$(CH_2)_2C{\equiv}CH$ (**13**)	0.05 (0.03−0.1)	0.04 (0.02−0.1)	0.1 (0.05−0.4)	inactive	substituted completely
$(CH_2)_3C{\equiv}CH$ (**14**)	inactive	12 (5−30)	inactive	2 (1−4)	not tested

[a] In mice, ED_{50} (95% confidence limits). [b] TF = tail-flick (morphine sulfate = 0.92 (0.89−4.14)). [c] PPQ = paraphenylquinone (morphine sulfate = 0.4 (0.2−0.8)). [d] HP = hot-plate (morphine sulfate = 3.1 (1.5−6.4)). [e] TF vs M = tail-flick vs morphine ED_{80} (naloxone hydrochloride = 0.04 (0.01−0.09)). [f] SDS = single-dose suppression study in morphine-dependent rhesus monkeys in withdrawal (morphine sulfate substituted completely); parenthesized numbers represent dose range in mg/kg. [g] Inactive = less than 70% of mice affected at 30 mg/kg. [h] CNS effects observed. [i] See ref 18. [j] See ref 20.

The (−)-(1*R*,5*R*,9*R*)-*N*-2-cyanoethyl (**3**) and -*N*-3-cyanopropyl (**4**) normetazocines had potent antinociceptive activity in the tail-flick, phenylquinone, and hot-plate assays (Table 4), and their effects in vivo could be ascribed to both their μ- and κ-opioid interactions. The tail-flick antinociceptive activity of the *N*-2-cyanoethyl compound **3** could be antagonized by nor-BNI, and the high AD_{50} which was shown by naloxone antagonism of the tail-flick antinociceptive activity of both **3** and **4** was characteristic for compounds with heterogeneous opioid interactions. As we previously observed with (−)-(1*R*,5*R*,9*R*)-*N*-benzylnormetazocine,[14] the (−)-*N*-cyanomethyl analogue **2** had considerably less in vitro and in vivo activity than might have been anticipated from the activity and potency of the (−)-*N*-allyl homologue **8**. Neither of the two most potent agonists, the (−)-*N*-2-cyanoethyl- and (−)-*N*-3-cyanopropyl-*N*-normetazocines, completely substituted for morphine in the single-dose suppression assay in monkeys (Table 4), which might indicate that they would have less physical

dependence potential in man than morphine. However, other CNS effects, observed with both compounds in the monkey, are likely to rule against human use.

As with the cyanoalkyl homologues **3**−**5**, the affinity of the (−)-(1*R*,5*R*,9*R*)-*N*-alkynyl and -alkenyl compounds for the μ-opioid receptor varied inversely with the distance between the nitrogen atom and the triple or double bond. Thus, the (−)-(1*R*,5*R*,9*R*)-*N*-but-2-ynylnormetazocine (**12**) and the *N*-allyl analogue **8** were both high-affinity ligands at the μ-receptor, and **12**, with increased steric hindrance around the triple bond caused by the additional methyl group, was much more selective for that receptor than **8**. (−)-(1*R*,5*R*,9*R*)-*N*-Allylnormetazocine (**8**) had 3-fold higher affinity at the κ- than μ-receptor. The (−)-(1*R*,5*R*,9*R*)-alkenyl series was also similar to the cyanoalkyl analogues in that a 5- or 10-fold decrease in μ- or κ-receptor affinity occurred as the unsaturation was moved more than three carbon atoms form the nitrogen atom, as seen with the hex-5-enyl (**11**) and pent-4-enyl (**10**) homologues in Table 2.

In the (−)-(1*R*,5*R*,9*R*)-alkenyl series, moving the unsaturation further than two carbon atoms away from the nitrogen atom reduced or eliminated opioid antagonist action in the mouse tail-flick vs morphine assay (compound **8** vs **9**−**11**, Table 4). A similar effect was seen in the *N*-alkyl series,[1] where increased chain length beyond the size of the *N*-propyl moiety appeared to cause a loss in antagonist potency. Thus, the (−)-but-3-enyl (**9**) and pent-4-enyl (**10**) analogues were 20 or 30 times less effective as opioid antagonists in the mouse tail-flick vs morphine assay than the (−)-*N*-allyl analogue **8**, and the (−)-hex-5-enyl homologue **11** had no opioid antagonist activity in that assay (Table 4).[1]

The double bond at the end of a long chain in the (−)-(1*R*,5*R*,9*R*)-alkenyl series appeared to increase antinociception a little beyond that found in a compound with a saturated six-carbon chain.[1] The (−)-hex-5-enyl analogue **11** is 4-fold more potent than the (−)-*N*-hexyl analogue in the mouse tail-flick assay (ED_{50} = 0.3 mg/kg for **11**, compared with 1.1 mg/kg for (−)-(1*R*,5*R*,9*R*)-*N*-hexylnormetazocine) and more than 5 times more potent in the hot-plate assay. The (−)-but-3-enyl analogue **9** (but not the intermediate-sized pent-4-enyl analogue **10**) also displayed potent antinociceptive action in the mouse tail-flick assay. (−)-(1*R*,5*R*,9*R*)-*N*-But-3-enylnormetazocine (**9**) was about 20 times more potent than morphine in the mouse tail-flick assay. Interestingly, (−)-*N*-allylnormetazocine (**8**) was essentially inactive in that assay, as well as the hot-plate assay. The weak antinociceptive effects of the *N*-pent-4-enyl analogue **10** were very different from the morphine-like antinociceptive activity found with the comparable saturated *N*-alkyl compound, (−)-*N*-pentylnormetazocine (mouse tail-flick ED_{50} = 1.3 mg/kg).[1]

In the (−)-(1*R*,5*R*,9*R*)-*N*-alkynyl series, the but-3-ynyl compound **13** was found to be a potent agonist in all three antinociception assays. In vivo opioid type tests[21] revealed that it was a nonselective μ-, κ-, and δ-opioid agonist (β-FNA AD_{50} = 0.6 (0.3−1.31) μg/brain, nor-BNI AD_{50} = 0.3 (0.1−0.9) mg/kg sc, and naltrindole AD_{50} = 3.1 (1.0−9.4) mg/kg sc). These values were consistent with the binding affinities shown for **13** in Table 2 (μ = 4.6, κ = 2.9, and δ = 9.9 nM, respectively). In sharp contrast, the but-2-ynyl (**12**) and pent-4-ynyl (**14**) analogues were essentially inactive as antinociceptives. However, compound **14** was found to be a surprisingly potent δ-opioid antagonist in the selective in vivo antagonist assay[22] (AD_{50} of **14** vs DPDPE (a δ-agonist) = 0.05 (0.02−0.11) mg/kg). It was considerably more efficacious than anticipated from its binding affinity with the δ-opioid receptor (137 nM). The pent-4-ynyl derivative **14** was not selective; it was also found to act as a μ- and κ-opioid antagonist. The binding affinity of **14** at μ- or κ-opioid receptors (K_i = 18 and 11 nM, respectively, Table 2) appeared to be more closely related to its efficacy as an antagonist at those receptors than it was at the δ-receptor (AD_{50} of **14** vs ED_{80} of morphine = 20 (1.1−3.7) mg/kg and AD_{50} of **14** vs ED_{80} of U69,593 = 1.2 (0.5−3.3) mg/kg, where morphine and U69,593 were the μ- and κ-agonists utilized). Unlike the alkenyl and alkynyl series, only one of the examined (−)-cyanoalkyl homologues, the *N*-5-cyanopentyl **6**, was found to have any opioid antagonist activity.

Among the (+)-(1*S*,5*S*,9*S*)-*N*-cyanoalkyl homologues, all but the (+)-cyanomethyl (**16**) and (+)-2-cyanoethyl (**17**) enantiomers interacted with the σ_1-binding site with reasonably high affinity ($K_i \sim$ 36 nM for the (+)-4-cyanobutyl (**19**) and (+)-5-cyanopentyl (**20**) to about 20 nM for the (+)-3-cyanopropyl (**18**) and (+)-6-cyanohexyl (**21**) homologues (Table 2)). A linear relationship between lipophilicity due to increasing chain length and affinity to the σ_1-binding site was not observed in this series of compounds.[1,23] Several (+)-alkenyl and (+)-alkynyl compounds had good affinity to the σ_1-binding site. The (+)-pent-4-enyl (**24**) and (+)-hex-5-enyl (**25**) homologues showed both high affinity (K_i = 2 and 1 nM, respectively) and good selectivity (σ_2/σ_1 = 1250 and 750, respectively) for that site. In the (+)-alkenyl series, lipophilicity (*N*-alkenyl chain length) and σ_1 affinity do appear to be linearly related. Some (−)-ligands were noted to interact with higher affinity (Tables 2 and 3) to the σ_2-site than any of the (+)-enantiomers (e.g., (−)-hex-5-enyl **11**, K_i = 84 nM, and (−)-pent-4-enyl **10**, K_i = 181 nM), but in both the (−)- and (+)-series all of the compounds showed higher affinity for the σ_1- than the σ_2-site.

Few of the (+)-(1*S*,5*S*,9*S*)-*N*-cyanoalkyl, -alkenyl, and -alkynyl homologues had opioid-like actions in vivo. Only the (+)-cyanomethyl (**16**), 6-cyanohexyl (**21**), and pent-4-ynyl (**28**) analogues showed any antinociceptive activity, and that activity could only be seen in the mouse PPQ assay (ED_{50} = 6.0 (4.2−8.4), 9.4 (3.3−27), and 14.8 (11−20) mg/kg, respectively), and only the allyl (**22**)[24] and but-2-ynyl (**26**) analogues displayed narcotic antagonist activity in the mouse tail-flick vs morphine assay (AD_{50} = 13.2 (6.6−26) and 6.7 (2.4−19), respectively). The compounds which had in vivo agonist or opioid antagonist activity had little or no affinity for any opioid receptor (K_i > 10000 nM), with the exception of the (+)-pent-4-ynyl compound **28** which had slight affinity for μ- and κ-receptors (K_i > 3500 and 300 nM, respectively). Conversely, none of the (+)-analogues which showed at least a little (<2000 nM) affinity for an opioid receptor (**17**−**20**, **24**, **25**, **27**, and **28**) displayed any in vivo activity.

Discussion

The effect of the position of a comparatively polar group like cyano, or of unsaturation (moieties with

double and triple bonds) in the side chain on the nitrogen atom of the 6,7-benzomorphans, was seen to be very variable. In the (−)-*N*-alkenyl series, (−)-*N*-allylnormetazocine is an opioid antagonist[24,25] (its antagonist activity in the tail-flick vs morphine assay was comparable to that of its saturated *N*-propyl relative[1]). As the distance between the double bond and the nitrogen atom increased, major changes were seen in receptor affinity and antinociceptive and opioid antagonist activity. When a triple bond replaces the double bond in the side chain, only a few qualitative changes were noted (compare **9** vs **13** and **10** vs **14**). When a cyano group is placed in the side chain, modifying, among other things, the polarity of that side chain, a qualitative change (compare **3** vs **9** and **13**) was initially observed, followed by a more substantive change when that substituent appeared further out on the side chain (**4** vs **10** and **14**); the 3-cyanopropyl analogue **4** was a potent agonist and both the pent-4-enyl (**10**) and pent-4-ynyl (**14**) analogues were weak agonists and weak antagonists. Movement of the double or triple bond away from the nitrogen atom appeared to increase antinociceptive activity and lessen opioid antagonist actions, although the effect was not linear and the change was seen to lessen (as in **10**) and then return (as in **11**). Thus, the position of the unsaturation in the *N*-substituent chain in the alkenyl, cyanoalkyl, and alkynyl series affected antinociception, opioid antagonist activity, and affinity for opioid receptors.

With the data that others and we have obtained, it is conceivable that sufficient information will eventually become available to gain insight into how the *N*-substituent affects the activity of opioid ligands and enable prediction of that activity. Since many opioids are known to selectively interact with a specific opioid receptor, notwithstanding the high homology which exists among the opioid receptors, it is likely that in vivo activity of an opioid is related to its opioid receptor affinity, as well as efficacy and, perhaps, one or more physicochemical parameters such as lipophilicity. The receptor affinity and efficacy of opioid ligands may eventually be rationalized through improved molecular modeling and docking.

Experimental Section

Melting points (uncorrected) were taken in a Thomas-Hoover capillary apparatus. Structures were consistent with mass and IR spectra. Optical rotations (Perkin-Elmer 141 digital polarimeter) were determined in MeOH. Elemental analyses were performed for C, H, and N, at Atlantic Microlabs, Inc., Atlanta, GA, and were within ±0.4% of theory. The bromoalkyl cyanides and alkenes are from the Aldrich Chemical Co., Milwaukee, WI.

General Procedure. 1*R*,5*R*,9*R*-(−)-2-(3-Cyanopropyl-5,9-dimethyl-2′-hydroxy-6,7-benzomorphan.[3] (−)-(1*R*,5*R*,-9*R*)-*N*-Normetazocine (**1**, R = H), 0.5 g (0.23 mmol), 0.37 g (0.240 mmol) of 3-bromopropionitrile, 0.7 g (large excess) of $KHCO_3$, 5 mL of THF and 1 mL of DMF (both solvents dried over K_2CO_3) were refluxed (stirring) for 5 h. The THF was evaporated in vacuo. The residue was treated with H_2O and Et_2O (or AcOEt), 2 extractions. The H_2O-washed, dried (over Na_2SO_4) extracts were evaporated in vacuo to give 0.8 g of compound **3** which in 7 mL of Me_2CO was acidified with 1 M ethereal HCl. Standing for 3−5 h at 25 °C and recrystallization from MeOH−Me_2CO gave 0.57 g of **3**·HCl: mp 220−222 °C.

In Vivo Assays. Mouse Opioid Agonist/Antagonist Evaluation. All animals received care according to *Guide for the Care and Use of Laboratory Animals*, U.S. Department of Health and Human Services, 1985. The facilities are certified by the American Association for the Accreditation of Laboratory Animal Care. These studies were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

General Methods. ICR male mice (Harlan Sprague−Dawley, Inc., Indianapolis, IN) weighing 20−30 g were used. Each animal was tested once only. All drugs were given by the subcutaneous (sc) route. At least 3 doses were tested, and 6−10 animals/dose were used. In the mouse antinociceptive studies, drugs were dissolved in distilled water or hydroxypropyl-β-cyclodextrin or a few drops of lactic acid in water. In the monkey studies, drugs were dissolved in sterile water for injection or hydroxypropyl-β-cyclodextrin aqueous solution.

Tail-Flick Agonist and Antagonist Tests. The procedures[26,27] and their modifications were previously described.[1] Mice were injected with test drug or vehicle and tested 20 min later. Antinociception was calculated as % MPE (percent maximum possible effect) = (test latency − control latency/(10 s − control latency) × 100 for each dose tested. Cutoff time was 10 s. In the naloxone antagonism test, naloxone was given 10 min before the test drug ED_{80} (dose producing 80% increase in MPE) was injected and latencies were measured 20 min later. For each point of the dose−response curve, percent antagonism was calculated as [1 − (naloxone + test drug MPE)/(test drug MPE ED_{80})] × 100.

Hot-Plate Test. A 1000-mL Pyrex beaker (bottom removed) was placed on the hot plate maintained at 56 °C. The test was initiated by placing a mouse in the specially designed beaker. This arrangement served to confine a mouse to a specific area of the hot plate. Each mouse was exposed to the hot plate for 2 trials spaced 5 min apart. Only mice that gave a control response latency in the range of 6−10 s on both trials served as subjects. Each subject received a dose of test drug and 30 min later was again tested on the hot plate. Activity was scored as positive if the mouse jumped, licked or shook its paws at least 5 s beyond its average control latency. Cutoff time was 15 s. Percent activity for each dose tested was calculated as (total number of mice scored as positive)/(total number tested) × 100.

Selective in Vivo Agonist and Antagonist Activity Determination.[21,22] Using the tail-flick or PPQ assay, selective opioid agonist activity was established in mice by giving β-FNA icv 4 h before challenge with an ED_{80} dose of a possible μ-agonist sc, nor-BNI was given sc 2 h before an ED_{80} of a suspected κ-agonist, which was also given sc, and naltrindole was given sc 20 min before a sc ED_{80} dose was given of a candidate δ-agonist. In vivo antagonist activity was determined by giving an ED_{80} dose of DPDPE icv 10 min before a sc ED_{80} dose of a purported δ-antagonist. The κ-antagonist profile of a drug was established by givng an ED_{80} dose of U69,-593 sc 20 min prior to the drug in question. The μ-antagonist behavior of a drug in vivo was determined by giving an ED_{80} dose of morphine sc 20 min before challenge with a drug that might have μ-antagonist properties.

Single-Dose Substitution Test in Rhesus Monkeys. The procedure was previously described.[1,28,29] The withdrawal signs were scored during each of five 30-min observation periods. The trained observer was "blind" regarding treatment assignments. At the end of the study, the data were grouped according to dose and drug and analyzed.

Precipitated Withdrawal Test in Rhesus Monkeys. This evaluation was conducted under the same conditions described above, except that the test compound in question was administered to monkeys 2−3 h after the last dose of morphine and challenged with naloxone hydrochloride (0.05 mg/kg sc). At the time of challenge the monkeys were not in withdrawal.

Statistical Analyses. MPE ED_{50}, ED_{80} or AD_{50} values were calculated according to the methods described by Litchfield and Wilcoxon.[30]

Receptor Binding Assays. σ binding assay: The σ_1 assay was carried out with 3 nM (+)-[^{3}H]pentazocine as the radioligand, according to the literature.[1] Homogenates of whole brains from male Hartley guinea pigs were used. The σ_2 assay was carried out with 5 nM [^{3}H]DTG as the radioligand and included 1 μM dextrallorphan to mask σ_1 binding. Rat liver membranes from male Sprague−Dawley rats were used according to the literature.[31] The K_i values were calculated from IC_{50} values using the Cheng−Prusoff equation[32] and the K_d values of (+)-[^{3}H]pentazocine (3.4 nM) and [^{3}H]DTG (17.9 nM) were obtained by Scatchard analysis and are the mean (±SEM) of three separate experiments.

Opioid binding assays: Ligand binding assays were determined for compounds at μ-, δ-, and κ-opioid receptors in monkey brain cortex according to the literature.[33] Membrane protein (ca. 300 μg) was incubated with an appropriate selective ligand ([^{3}H]DAMGO, [^{3}H]DPDPE, or [^{3}H]U69,593) at its K_d concentration (0.57, 2.1, or 0.95 nM, respectively). All values are means ± SEM of at least three experiments except when a percentage value is given, then $n = 2$. Data were analyzed using nonlinear curve fitting with GraphPad Prism (GraphPad, San Diego, CA).

Acknowledgment. We thank Larry Hughes, Zhen Ji, and Susan Scates (all from Virginia Commonwealth University) for their expert assistance and Hui-fang Song for opioid binding (University of Michigan). We are indebted to Dr. Paul Hillery, NIDA, and to Drs. F. Ivy Carroll and George Brine, RTI, for generous supplies of α-(−)- and (+)-*N*-normetazocine. This research was supported in part by the National Institute on Drug Abuse, Bethesda, MD [DA 5-8059 (Virginia Commonwealth University) and DA 00254 (University of Michigan)]. We also thank Dr. Andrew Coop, Department of Pharmaceutical Sciences, University of Maryland School of Pharmacy, for discussions and suggestions. The pharmacological evaluation of the compounds was carried out under the auspices of the Drug Evaluation Committee (Dr. Andrew Coop, Biological Coordinator) of the College on Problems of Drug Dependence.

Supporting Information Available: Analytical data for compounds **2**−**7**, **9**−**14**, **16**−**21**, and **23**−**28**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) May, E. L.; Aceto, M. D.; Bowman, E. R.; Bentley, C.; Martin, B. R.; Harris, L. S.; Medzihradsky, F.; Mattson, M. V.; Jacobson, A. E. Antipodal α-N-Alkyl (Methyl-Decyl)-N-Normetazocines (2′-Hydroxy-5,9α-Methyl-6,7-Benzomorphans): In Vitro and In Vivo Properties. *J. Med. Chem.* **1994**, *37*, 3408−3418.

(2) Filizola, M.; Laakkonen, L.; Loew, G. H. 3D Modeling, Ligand Binding and Activation Studies of the Cloned Mouse Delta, Mu and Kappa Opioid Receptors. *Protein Eng.* **1999**, *12*, 927−942.

(3) Filizola, M.; Carteni-Farina, M.; Perez, J. J. Molecular Modeling Study of the Differential Ligand−Receptor Interaction at the Mu, Delta and Kappa Opioid Receptors. *J. Comput.-Aided Mol. Des.* **1999**, *13*, 397−407.

(4) Portoghese, P. S. Impact of Recombinant DNA Technology and Molecular Modeling on the Practice of Medicinal Chemistry: Structure−Activity Analysis of Opioid Ligands. *Am. J. Pharm. Ed.* **1999**, *63*, 342−347.

(5) Subramanian, G.; Paterlini, M. G.; Portoghese, P. S.; Ferguson, D. M. Molecular Docking Reveals a Novel Binding Site Model for Fentanyl at the Mu-Opioid Receptor. *J. Med. Chem.* **2000**, *43*, 381−391.

(6) Atsumi, T.; Kobayashi, K.; Takebayashi, Y.; Yamamoto, H. Derivatives of 2-Substituted-Cyanoalkylbenzomorphan. Sumitomo Chemical Co., Ltd., Japan Patent JP 49072261, 1974.

(7) Atsumi, T.; Kobayashi, K.; Takebayashi, Y.; Yamamoto, H. Analgesic N-(ω-Cyanoalkyl)-6,7-Benzomorphans. Sumitomo Chemical Co., Ltd., Germany Patent No. 22224596, 1972.

(8) Jacobson, A. E.; Rice, K. C.; Reden, J.; Lupinacci, L.; Brossi, A.; Streaty, R. A.; Klee, W. A. Paradoxical Effects of N-Cyanoalkyl Substituents Upon the Activities of Several Classes of Opioids. *J. Med. Chem.* **1979**, *22*, 328−331.

(9) Iwamoto, E. T.; Martin, W. R. Multiple Opioid Receptors. *Med. Res. Rev.* **1981**, *1*, 411−440.

(10) Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. The Effect of Morphine and Nalorphine-Like Drugs in the Nondependent and Morphine-Dependent Chronic Spinal Dog. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517−532.

(11) Quirion, R.; Bowen, W. D.; Itzhak, Y.; Junien, J. L.; Musacchio, J. M.; Rothman, R. B.; Su, T. P.; Tam, S. W.; Taylor, D. P. A Proposal for the Classification of Sigma Binding Sites. *Trends Pharmacol. Sci.* **1992**, *13*, 85−86.

(12) Quirion, R.; Chicheportiche, R.; Contreras, P. C.; Johnson, K. M.; Lodge, D.; Tam, S. W.; Woods, J. H.; Zukin, S. R. Classification and Nomenclature of Phencyclidine and Sigma Receptor Sites. *Trends Neurosci.* **1987**, *10*, 444−446.

(13) Brady, K. T.; Balster, R. L.; May, E. L. Stereoisomers of N-Allylnormetazocine: Phencylidine-Like Behavioral Effects in Squirrel Monkeys and Rats. *Science* **1982**, *215*, 178−180.

(14) May, E. L.; Jacobson, A. E.; Mattson, M. V.; Coop, A.; Aceto, M. D.; Bowman, E. R.; Traynor, J. R.; Woods, J. H.; Harris, L. S. Synthesis, In Vitro, and In Vivo Activity of N-*p*-Substituted-Benzyl (−)- and (+)-N-Normetazocines, (−)- and (+)-N-Normorphinans, N-Norketobemidones, and (−)-N-nor-4,5-Epoxymorphinans. *Med. Chem. Res.* **1998**, *8*, 311−321.

(15) Carroll, F. I.; Abraham, P.; Parham, K.; Bai, X.; Zhang, X.; Brine, G. A.; Mascarella, S. W.; Martin, B. R.; May, E. L.; Sauss, C.; Dipaolo, L.; Wallace, P.; Walker, J. M.; Bowen, W. D. Enantiomeric N-Substituted N-Normetazocines − a Comparative Study of Affinities at σ, PCP, and μ Opioid Receptors. *J. Med. Chem.* **1992**, *35*, 2812−2818.

(16) Bowen, W. D.; Vilner, B. J.; Williams, W.; Bertha, C. M.; Kuehne, M. E.; Jacobson, A. E. Ibogaine and Its Congeners Are σ2 Receptor-Selective Ligands with Moderate Affinity. *Eur. J. Pharmacol.* **1995**, *279*, R1−R3.

(17) Archer, S.; Albertson, N. F.; Harris, L. S.; Pierson, A. K.; Bird, J. G. Pentazocine. Strong Analgesics and Analgesic Antagonists in the Benzomorphan Series. *J. Med. Chem.* **1964**, *7*, 123−127.

(18) Aceto, M. D.; Harris, L. S.; May, E. L. Dependence Studies of New Compounds in the Rhesus Monkey, Rat and Mouse. *Problems of Drug Dependence 1982*; NIDA Research Monograph 43; NIDA: Washington, DC, 1983; pp 399−456.

(19) Aceto, M. D.; May, E. L.; Harris, L. S.; Jacobson, A. E.; Awaya, H. Agonist-Antagonist Actions and Stereoselectivity of Optical Pairs of Some N-Substituted α-N-Normetazocines. *J. Pharm. Sci.* **1984**, *73*, 1864−1866.

(20) de Costa, B.; George, C.; Rothman, R. B.; Jacobson, A. E.; Rice, K. C. Synthesis and Absolute Configuration of Optically Pure Enantiomers of a Kappa-Opioid Receptor Selective Agonist. *FEBS Lett.* **1987**, *223*, 335−339.

(21) Aceto, M. D.; Harris, L. S.; Bowman, E. R. Etorphines: Mu-Opioid Receptor-Selective Antinociception and Low Physical Dependence Capacity. *Eur. J. Pharmacol.* **1997**, *338*, 215−223.

(22) Aceto, M. D.; Bowman, E. R.; Harris, L. S.; May, E. L. Dependence Studies of New Compounds in the Rhesus Monkey, Rat and Mouse. *Problems of Drug Dependence 1995*; NIDA Research Monograph 162; NIDA: Washington, DC, 1996; pp 408−451.

(23) Kaiser, C.; Pontecorvo, M. J.; Mewshaw, R. E. Sigma Receptor Ligands: Function and Activity. *Neurotransmissions*; Research Biochemicals Inc., 1991.

(24) Swain, H. H.; Villarreal, J. E.; Seevers, M. H. Evaluation of New Compounds for Morphine-Like Physical Dependence in the Rhesus Monkey. *Committee on Problems of Drug Dependence, 1973*; National Academy of Sciences: Washington, DC, 1973; pp 693−713.

(25) Aceto, M. D.; May, E. L. Antinociceptive Studies of Optical Isomers of *N*-Allylnormetazocine SKF10,047. *Eur. J. Pharmacol.* **1983**, *91*, 267−272.

(26) D′Amour, F. E.; Smith, D. L. A Method for Determining Loss of Pain Sensation. *J. Pharmacol. Exp. Ther.* **1941**, *72*, 74−79.

(27) Dewey, W. L.; Harris, L. S. Antinociceptive Activity of the Narcotic Antagonists Analogues and Antagonistic Activity of Narcotic Analgesics in Rodents. *J. Pharmacol. Exp. Ther.* **1971**, *179*, 652−659.

(28) Aceto, M. D.; Flora, R. E.; Harris, L. S. Caffeine Elicited Withdrawal Signs in Morphine-Dependent Rhesus Monkeys. *Eur. J. Pharmacol.* **1978**, *50*, 203−207.

(29) Aceto, M. D.; Flora, R. E.; Harris, L. S. The Effects of Naloxone and Nalorphine During the Development of Morphine Dependence in Rhesus Monkeys. *Pharmacology* **1977**, *15*, 1−9.

(30) Litchfield, J. T.; Wilcoxon, F. A Simplified Method of Evaluating Dose−Effect Experiments. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99−113.

(31) Hellewell, S. B.; Bruce, A.; Feinstein, G.; Orringer, J.; Williams, W.; Bowen, W. D. Rat Liver and Kidney Contain High Densities of Sigma(1) and Sigma(2) Receptors: Characterization by Ligand Binding and Photoaffinity Labeling. *Eur. J. Pharmacol.* **1994**, *268*, 9−18.

(32) Cheng, Y.-C.; Prusoff, W. H. Relationship between the Inhibition Constant (*Ki*) and the Concentration of Inhibitor Which Causes 50 Percent Inhibition (IC_{50}) of an Enzymatic Reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099−3108.

(33) Emmerson, P. J.; Liu, M. R.; Woods, J. H.; Medzihradsky, F. Binding Affinity and Selectivity of Opioids at Mu, Delta and Kappa Receptors in Monkey Brain Membranes. *J. Pharmacol. Exp. Ther.* **1994**, *271*, 1630−1637.

JM000317+