J. Med. Chem. 1992, 35, 2826–2835

Probes for Narcotic Receptor Mediated Phenomena. 18.[1] Epimeric 6α- and 6β-Iodo-3,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinans as Potential Ligands for Opioid Receptor Single Photon Emission Computed Tomography: Synthesis, Evaluation, and Radiochemistry of [^{125}I]-6β-Iodo-3,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan

Brian R. de Costa,[†] Michael J. Iadarola,[‡] Richard B. Rothman,[⊥] Karen F. Berman,[§] Clifford George,[∥] Amy H. Newman,[°] Artin Mahboubi,[†] Arthur E. Jacobson,[†] and Kenner C. Rice*,[†]

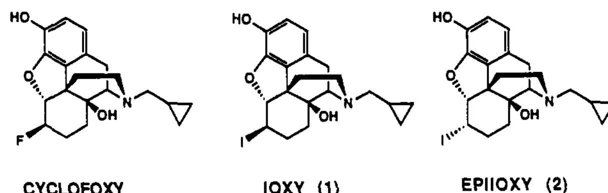
Room B1-23, Bldg 8, Laboratory of Medicinal Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, 9000 Rockville Pike, Bethesda, Maryland 20892, Neurobiology and Anesthesiology Branch, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20892, Clinical Brain Disorders Branch, National Institute of Mental Health, Washington, D.C. 20032, Naval Research Laboratory, Code 6030, Washington, D.C. 20375, Laboratory of Clinical Psychopharmacology, National Institute on Drug Abuse, Addiction Research Center, Baltimore, Maryland 21224, and Psychobiology Laboratory, National Institute on Drug Abuse, Addiction Research Center, Baltimore, Maryland 21224. *Received February 28, 1992*

The epimeric 6β- and 6α-iodo-3,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinans (1, ioxy) and (2, epioxy), respectively, were each synthesized in five steps starting with naltrexone. The configuration of the 6-iodo group of 1 was unequivocally determined to be β-based on single crystal X-ray analysis of its precursor 3-acetoxy-6β-iodo-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (10). Both 1 and 2 as well as their corresponding 3-O-acetates 10 and 11 were found to readily cross the blood–brain barrier and completely reverse the analgesic effects of a 10 mg/kg intraperitoneal dose of morphine sulfate as determined by the paw withdrawal latency test. Compounds 1 and 2 were found to bind with high affinity to μ, δ, and κ receptors in vitro. In general, 1 and 2 exhibited higher affinity for μ and κ receptors than naltrexone while the 6β-iodo epimer 1 (ioxy) was more potent than its epimer 2. In a comparison of the 6β-halogen substituent on binding affinity across opioid receptor subtypes, it was generally found that I > Br > F. On the basis of the results of in vitro and in vivo testing, 1 was selected as a target for radioiodination and evaluation as a potential single photon emission computed tomography imaging agent for opioid receptors. Carrier-free [^{125}I]-1 was synthesized in near quantitative yield by the sequence of reaction of excess 3-acetoxy-6α-[[(trifluoromethyl)sulfonyl]oxy]-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (8) with anhydrous Na^{125}I in dry acetonitrile for 90 min at 76 °C followed by deacetylation of the product with 1:1 aqueous ammonia/acetonitrile at 25 °C. The potential of [^{125}I]-1 as an in vivo imaging agent for opioid receptors is evaluated and discussed.

Introduction

Single photon emission computed tomography (SPECT)[2-5] and positron emission tomography (PET)[2-6] are related, noninvasive imaging techniques which are applicable to in vivo measurement of biochemical function in animals and conscious humans. These techniques have been used to measure nonspecific markers of local neuronal activity, such as regional cerebral blood flow[3] and the local cerebral metabolic rate for glucose.[7] During the last decade, however, extension of the SPECT and PET techniques to investigate central nervous system (CNS) active drugs has provided a breakthrough in the study of neurotransmitter systems.[2-6] Among the many biochemical and pharmacological approaches utilized in the study of the CNS only these two techniques are applicable to the visualization and quantitation of drug-receptor distribution in the CNS of living humans.[3] Herein, we report synthesis, pharmacological characterization, and radiolabeling of the narcotic antagonist ioxy (1) as a potential SPECT imaging agent for human opioid receptors.

The opioid receptor-endorphin system[8,9] consists of numerous endogenous opioid peptides which subserve three well-defined μ-, δ-, and κ-receptor subtypes. In addition, there is also strong evidence supporting the existence of a μ–δ-complex.[10] This system is thought to be



involved in the regulation of numerous physiological processes[9,11] including immune function and the perception

[†] National Institute of Diabetes and Digestive and Kidney Diseases, NIH.

[‡] National Institute of Dental Research, NIH.

[§] Clinical Brain Disorders Branch, National Institute of Mental Health.

[∥] Naval Research Laboratory.

[⊥] Laboratory of Clinical Psychopharmacology, National Institute on Drug Abuse.

[°] Psychobiology Laboratory, National Institute on Drug Abuse.

(1) de Costa, B. R.; Rothman, R. B.; Bykov, V.; Band, L.; Pert, A.; Jacobson, A. E.; Rice, K. C. Probes for Narcotic Receptor Mediated Phenomena. 17. Synthesis and Evaluation of a Series of U50,488 Related Isothiocyanate Derivatives as Opioid Receptor Affinity Ligands. *J. Med. Chem.* **1990**, *33*, 1171–1176.
(2) Diksic, M.; Reba, R. C. *Radiopharmaceuticals and Brain Pathology Studies with PET and SPECT*; CRC Press, Inc.: Boca Raton, FL, 1991.
(3) Andreasen, N. C.; Brain Imaging: Applications in Psychiatry. *Science* **1988**, *239*, 1381–1388.
(4) Frost, J. J.; Wagner, H. N. *Quantitative Imaging. Neuroreceptors, Neurotransmitters and Enzymes*; Raven Press: New York, 1990.
(5) Frost, J. J. Imaging Neuronal Biochemistry by Emission Computed Tomography. Focus on Neuroreceptors. *Trends Pharmacol. Sci.* **1986**, 490–496.
(6) Phelps, M. E.; Maziotta, J. C. Positron Emission Tomography: Human Brain Function and Biochemistry. *Science* **1985**, *228*, 799–809.
(7) Cunningham, V. J.; Cremer, J. E. Current Assumptions Behind the Use of PET Scanning for Measuring Glucose Utilization in Brain. *Trends Neurosci.* **1985**, *8*, 96–99.
(8) Robson, L. E.; Patterson, S. J.; Kosterlitz, H. W. Opiate Receptors. *Handbk Psychopharmacol.* **1983**, *17*, 13–80.
(9) Herz, A. The Multiplicity of Opioid Receptors and Their Functional Significance. In *Trends in Medicinal Chemistry*; Mutschler, E., Winterfeldt, E., Eds., VCH Verlagsgesellschaft: Weinheim, 1987; pp 337–350.

This article not subject to U.S. Copyright. Published 1992 by the American Chemical Society

of pain, pleasure, and mood. Evidence has been presented for heterogeneity of μ-,[12] δ-,[13-17] and κ-receptor[18] subtypes. As one line of investigation aimed at gaining further insight into the structure and function of the opioid receptor-endorphin system, we introduced[19] (−)-[^{18}F]cyclofoxy as a PET ligand and presented the first definitive PET images showing opioid receptor occupancy in the primate brain. In this 1984 study, we found robust accumulation of cyclofoxy in opioid-receptor-rich brain regions such as caudate and thalamus which was rapidly displaced by pharmacologically relevant doses of the narcotic antagonist naloxone but not by saline or equal doses of the opioid receptor inert (+)-enantiomer of naloxone,[20] now available[21] by the NIH Opiate Total Synthesis.[22] In later studies, we reported the synthesis of unlabeled (−)-cyclofoxy,[23] (−)-[^{3}H]cyclofoxy,[24] and detailed radiochemical synthesis of (−)-[^{18}F]cyclofoxy.[25] Other studies showed that (−)-cyclofoxy was a potent narcotic antagonist[26] which was suitable[27] for imaging human opioid receptors, and that the opioid receptor inert[28] (+)-cyclofoxy[29,30] when appropriately tritiated[31-34] is a valuable adjunct in the

(10) (a) Holaday, J. W.; Porreca, F.; Rothman, R. B. Functional coupling among opioid receptor types: Implications for anesthesiology. In: Opioids in Anesthesia II; Estafanous, F. G., Ed.; Butterworth Publishers: 1991; pp 50–60. (b) Rothman, R. B.; Holaday, J. W.; Porreca, F.; Allosteric coupling among opioid receptors: Evidence for an opioid receptor complex. In: Handbook of Experimental Pharmacology; Herz, A., Ed.; "Opioids" Part I. In press, 1992.

(11) Olson, G. A.; Olson, R. D.; Kastin, A. J. Endogenous Opiates: 1987. *Peptides* **1989**, *10*, 205–236.

(12) (a) Itzhak, Y. Multiple Opioid Binding Sites. In *The Opiate Receptors*; Pasternak, G. W., Ed.; The Humana Press: Clifton, NJ, 1988; pp 95–142. (b) Rothman, R. B.; Jacobson, A. E.; Rice, K. C.; Herkenham, M. Autoradiographic evidence for two classes of mu binding sites in rat brain using [^{125}I]FK33824. *Peptides* **1987**, *8*, 1015–1021. (c) Rothman, R. B.; Bykov, V.; Long, J. B.; Brady, L. S.; Jacobson, A. E.; Rice, K. C.; Holaday, J. W. Chronic administration of morphine and naltrexone up-regulate mu opioid binding sites labeled by [^{3}H]DAGO: Further evidence for two binding sites. *Eur. J. Pharmacol.* **1989**, *160*, 71–82.

(13) Xu, H.; Ni, Q.; Jacobson, A. E.; Rice, K. C.; Rothman, R. B. Preliminary Ligand Binding Data for Subtypes of the Delta Opioid Receptor in Rat Brain membranes. *Life Sci.* **1991**, *49*, PL 141–146.

(14) Jiang, Q.; Bowen, W. D.; Mosberg, H. I.; Rothman, R. B.; Porreca, F. Opioid Agonist and Antagonist Antinociceptive Properties of Enkephalin: Selective Actions at the $Delta_{noncomplexed}$ Site. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 636–641.

(15) Jiang, Q.; Takemori, A. E.; Sultana, M.; Portoghese, P. S.; Bowen, W. D.; Mosberg, H. I.; Porreca, F. Differential Antagonism of Opioid Delta Antinociception by [D-Ala^{2},Leu^{5},Cys^{6}]-enkephalin and Naltrindole 5′-Isothiocyanate: Evidence for Delta Receptor Subtypes. *J. Pharmacol. Exp. Ther.* **1991**, *257*, 1069–1075.

(16) Mattia, A.; Vanderah, T.; Mosberg, H. I.; Omnaas, J. R.; Bowen, W. D.; Porreca, F. Pharmacological Characterization of [D-Ala^{2},Leu^{5},Ser^{6}]enkephalin (DALES) Antinociceptive Actions at the $Delta_{noncomplexed}$ Opioid Receptor. *Eur. J. Pharmacol.* **1991**, *192*, 371–376.

(17) Mattia, A.; Vanderah, T.; Mosberg, H. I.; Porreca, F. Lack of Antinociceptive Cross-tolerance Between [D-Pen^{2},D-Pen^{5}]-enkephalin and [D-Ala^{2}]deltorphin II in Mice: Evidence for Delta Receptor Subtypes. *J. Pharmacol. Exp. Ther.* In press, 1991.

(18) Rothman, R. B.; Bykov, V.; de Costa, B.; Jacobson, A. E.; Rice, K. C.; Brady, L. S. Interaction of Endogenous Opioid Peptides and Other Drugs with Four Kappa Sites in Guinea Pig Brain. *Peptides* **1990**, *11*, 311–331.

(19) Pert, C. B.; Danks, J. A.; Channing, M. A.; Eckelman, W. C.; Larson, S. M.; Bennett, J. M.; Burke, T. R., Jr.; Rice, K. C. [^{18}F]-3-Acetylcyclofoxy: A Useful Probe for Visualization of Opiate Receptors in Living Animals. *Fed. Eur. Biochem. Soc.* **1984**, *177*, 281–286.

(20) Iijima, I.; Minamikawa, J.-I.; Jacobson, A. E.; Brossi, A.; Rice, K. C.; Klee, W. Studies in the (+)-Morphinan Series. V. Synthesis and Biological Properties of (+)-Naloxone. *J. Med. Chem.* **1978**, *21*, 398–400.

(21) Rice, K. C. The Development of a Practical Total Synthesis of Natural and Unnatural Codeine, Morphine and Thebaine. In: *The Chemistry and Biology of Isoquinoline Alkaloids*; J. D. Phillipson, M. F. Roberts, M. H. Zenk, Eds.; Springer-Verlag: New York, 1985; pp 191–203.

(22) Unnatural (+)-naloxone, (+)-morphine, and (+)-codeine are available at no charge to qualified investigators from the National Institute on Drug Abuse Drug Supply System. For further information, contact Dr. Robert L. Walsh, Research Technology Branch, NIDA, Rm 10A-13, Parklawn Bldg., 5600 Fishers Lane, Rockville, MD 20857.

(23) Burke, T. R., Jr.; Rice, K. C.; Pert, C. B. Synthesis of 17-Methyl- and 17-Cyclopropylmethyl-3,14-dihydroxy-4,5-epoxy-6β-fluoromorphinans (Foxy and Cyclofoxy) as Model Opioid Ligands Suitable for Positron Emission Transaxial Tomography. *Heterocycles* **1985**, *23*, 99–106.

(24) Ostrowski, N. L.; Burke, T. R., Jr.; Rice, K. C.; Pert, A.; Pert, C. B. The pattern of [^{3}H]cyclofoxy retention in rat brain after in vivo injection corresponds to the in vitro opiate receptor distribution. *Brain Res.* **1987**, *402*, 257–286.

(25) Channing, M. A.; Eckelman, W. C.; Bennett, J. M.; Burke, T. R., Jr.; Rice, K. C. Radiosynthesis of [^{18}F]-3-Acetylcyclofoxy, A High Affinity Opiate Antagonist. *Int. J. Appl. Radiat. Isot.* **1985**, *36*, 429–433.

(26) Aceto, M. D.; Bowman, E. R.; Harris, L. S.; May, E. L. Dependence Studies of New Compounds in the Rhesus Monkey, Rat and Mouse. In *Problems of Drug Dependence, 1986*; Harris, L. S., Ed.; National Institute on Drug Abuse Research Monograph 76; U.S. Government Printing Office: Washington, DC, 1987; pp 392–395, 422–423.

(27) Carson, R.; Channing, M.; Nordahl, T.; Gross, M.; Hauck-Newman, A.; Simpson, N.; Finn, R.; Pert, C.; Rice, K.; Blasberg, R.; Larson, S. F-18 cyclofoxy PET Imaging in Man. *J. Nucl. Med.* **1988**, *29*, 796.

(28) Rothman, R. B.; Bykov, V.; Reid, A.; de Costa, B. R.; Newman, A. H.; Jacobson, A. E.; Rice, K. C.: A Brief Study of the Selectivity of Norbinaltorphimine, (−)-Cyclofoxy and (+)-Cyclofoxy Among Opioid Receptors In vitro. *Neuropeptides* **1988**, *12*, 181–187.

(29) Newman, A. H.; Channing, M. A.; Finn, R. D.; Dunn, B.; Simpson, N.; Carson, R. E.; Ostrowski, N. L.; Cohen, R. M.; Burke, T. R., Jr.; Larson, S. M.; Rice, K. C. Ligands for Imaging Opioid Receptors in Conscious Humans. In *Problems of Drug Dependence, 1988*; L. S. Harris, Ed.; NIDA Research Monograph 90; U.S. Government Printing Office: Washington, DC, 1989; pp 117–121.

(30) Newman, A. H.; et al. Unpublished results.

(31) Newman, A. H.; Ostrowski, N.; Channing, M. A.; Rice, K. C. (+)- and (−)-Cyclofoxy: Application to The Quantitation of Opiate Receptors In Vivo. *Abs. Soc. Neurosci.* **1989**, *15*, 703, Abstract 285.8.

(32) Kawai, R.; Sawada, Y.; Channing, M.; Hauck-Newman, A.; Rice, K.; Blasberg, R. Kinetic Analysis of the Opiate Antagonist Cyclofoxy in Rat Brain: Simultaneous Infusion of Active and Inactive Enantiomers. *J. Pharmacol. Exp. Ther.* **1990**, *255*, 826–835.

(33) Kawai, R.; Sawada, Y.; Channing, M.; Hauck-Newman, A.; Rice, K. C.; Blasberg, R. G. BBB Transport and Rapid Tissue Binding of the Opiate Antagonist Cyclofoxy: Comparison of Receptor Binding and Receptor Inert Enantiomers. *Am. J. Physiol.* **1990**, *259* (*Heart Circ. Physiol. 28*, H1278–1287).

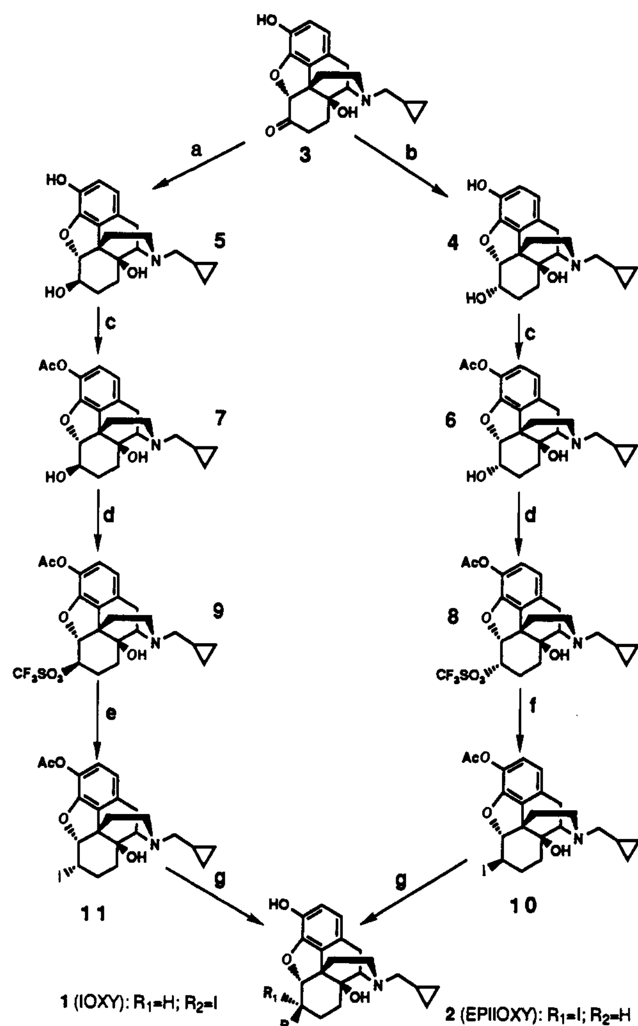
(34) Kawai, R.; Carson, R. E.; Dunn, B.; Newman, A. H.; Rice, K. C.; Blasberg, R. G.; Regional Brain Measurements of B_{max} and K_D with the Opiate Antagonist Cyclofoxy: Equilibrium Studies in the Conscious Rat. *J. Cereb. Blood Flow Metab.* **1991**, *11*, 529–544.

study of (-)-cyclofoxy.[31-35] Opioid receptor imaging has also been reported with the non-subtype-selective antagonist [^{11}C]diprenorphine[36,37] and the μ opioid receptor agonist ligand [^{11}C]carfentanyl,[38,39] which was employed to show increased μ-opioid receptor binding in human temporal lobe epilepsy.[40] A similar study with (-)-[^{18}F]-cyclofoxy did not detect such changes in binding.[41]

A number of other studies[5,42,43] have shown that the opioid receptor-endorphin system may be altered in drug-treated and pathological conditions as a result of changes in the opioid receptor density and affinity. PET and SPECT uniquely offer the ability to quantitate receptor density and affinity of human opioid receptors in their native state, and thus offer considerable advantages over tissue homogenization methods which can alter biochemical properties of receptors and necessarily must rely on human autopsy tissue. One goal of our PET and SPECT program is to develop clinical correlates of receptor function with human disease states involving abnormalities in the opioid receptor–endorphin system. In addition, the effects of drug treatment or abuse on the opioid receptor system could be monitored. Such studies require quantitation of opioid receptor affinity and density in vivo in normal and abnormal subjects. The recently reported[44] in vivo Scatchard analysis of [^{18}F]cyclofoxy binding using PET in the rhesus monkey brain constitutes a significant advance in this direction. With PET, the short half-life of commonly used positron-emitting radioisotopes such as ^{11}C ($t_{0.5}$ = 20 min) and ^{18}F ($t_{0.5}$ = 109 min) allows multiple experiments to be performed with the same subject. A number of positron-emitting radionuclides are available for different PET applications and through such applications the study of receptors in normal and disease states can provide a quantitative marker for certain psychiatric disorders.[3] The development of PET and SPECT ligands for drug-receptor quantitation is a major undertaking and the considerations in the development of such ligands have been perceptively reviewed.[45]

In SPECT studies, a ^{123}I-labeled form of a drug or ligand is usually employed. This differs from positron-emitting ligands in that it emits single photons, has a longer half-life (13.2 h), and does not require generation by an on-site

Scheme I.[a] Synthesis of Epimeric 6-Iodo-6-deoxynaltrexones 1 and 2



[a] (a) formamidinesulfinic acid, aqueous NaOH, 80–85 °C; (b) (i) KH, THF, (ii) K-Selectride, 5 → 25 °C; (c) Ac_2O, aqueous $NaHCO_3$; (d) $(CF_3SO_2)_2O$, *N*-methylmorpholine, $CHCl_3$; (e) $Et_4N^+I^-$, CH_3CN, 80 °C; (f) $Et_4N^+I^-$, CH_3CN, -10 → 20 °C; (g) concentrated aqueous NH_3 solution, room temperature, THF/MeOH.

(35) Sawada, S.; Kawai, R.; McManaway, M.; Otsuki, H.; Rice, K. C.; Patlak, C. S.; Blasberg, R. Kinetic Analysis of Transport and Opioid Receptor Binding of ^{3}H-(-)-Cyclofoxy in Rat Brain In Vivo: Implications for Human Studies. *J. Cereb. Blood Flow Metab.* **1991**, *11*, 183–203.
(36) Jones, A. K. P.; Luthra, S. K.; Maziere, B.; Pike, V. W.; Loc'h, C.; Crouzel, C.; Syrota, A.; Jones, T. Regional Cerebral Opioid Receptor Studies with [^{11}C]Diprenorphine in Normal Volunteers. *J. Neurosci. Methods* **1988**, *23*, 121.
(37) Frost, J. J.; Mayberg, H. S.; Sadzot, B.; Dannals, R. F.; Lever, J. R.; Ravert, H. T.; Wilson, A. A.; Wagner, H. N., Jr.; Links, J. M. Comparison of [^{11}C]Diprenorphine and [^{11}C]Carfentanyl Binding to Opiate Receptors in Humans by Positron Emission Tomography. *J. Cereb. Blood Flow Metab.* **1990**, *10*, 484–492.
(38) Frost, J. J.; Wagner, H. N.; Dannels, R. F.; Ravert, H. T.; Links, J. M.; Wilson, A. A.; Burns, H. D.; Wong, D. F.; McPherson, R. W.; Rosenbaum, A. E.; Kuhar, M. J.; Snyder, S. H. Imaging Opiate Receptors in the Human Brain by Positron Emission Tomography. *J. Comput. Assist. Tomogr.* **1985**, *9*, 231–236.
(39) Frost, J. J.; Douglass, K. H.; Mayberg, H. S.; Dannels, R. F.; Links, J. M.; Wilson, A. A.; Ravert, H. T.; Crozier, W. C.; Wagner, H. N., Jr. Multicompartment Analysis of [^{11}C]Carfentanyl Binding to Opiate Receptors in Man Measured by Positron Emission Tomography. *J. Cereb. Blood Flow Metabol.* **1989**, *9*, 398–409.
(40) Frost, J. J.; Mayberg, H. S.; Fisher, R. S.; Douglass, K. H.; Dannals, R. F.; Links, J. M.; Wilson, A. A.; Ravert, H. T.; Rosenbaum, A. E.; Snyder, S. H.; Wagner, H. N., Jr. Mu-opiate Receptors Measured by Positron Emission Tomography are Increased in Temporal Lobe Epilepsy. *Ann. Neurol.* **1988**, *23*, 231–237.
(41) Thedore, W. H.; Carson, R. E.; Andreasen, P.; Zametkin, A.; Blasberg, R.; Leiderman, D.; Cohen, R.; Rice, K.; Newman, A.; Dunn, B.; Simpson, N.; Herscovitch, P. PET Imaging of Opiate Receptor Binding in Human Epilepsy Using ^{18}F-cyclofoxy. *Arch. Neurol.* In press, 1992.
(42) Blanchard, S. G.; Chang, K.-J. Regulation of Opioid Receptors. In *The Opiate Receptors*; Pasternak, G. W., Ed.; The Humana Press: Clifton, NJ, 1988; pp 425–439.
(43) Smith, A. P.; Law, P.-Y.; Loh, H. H. Role of Opioid Receptors in Narcotic Tolerance/Dependence. In *The Opiate Receptors*; Pasternak, G. W., Ed.; The Humana Press: Clifton, NJ, 1988; pp 441–485.
(44) Carson, R.; Doudet, D.; Channing, M.; Dunn, B.; Der, M.; Newman, A.; Rice, K.; Cohen, R. Equilibrium Measurement of B_{max} and K_D of the Opiate Antagonist ^{18}F-cyclofoxy with PET: Pixel-by-pixel Analysis. *J. Cereb. Blood Flow Metab.* **1991**, *11*, S618.
(45) Eckelman, W. C.; Gibson, R. E. The Path from In Vitro Autoradiography to Ex Vivo Autoradiography to "In Vivo Autoradiography" by External Imaging. In *Using Autoradiography and Correlative Imaging In Vivo and In Vivo*; Stumpf, W., Soloman, H., Eds.; Academic Press: New York. In press, 1992.

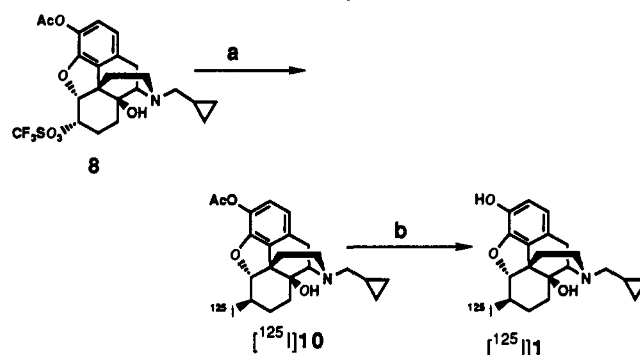
cyclotron as do positron-emitting radionuclides. The longer half-life of ^{123}I offers more time for observation of the subjects, and often provides sufficient time for clearance of nonspecific labeling where this is a problem. The resolution (7–8 mm) offered by recent-generation SPECT scanners is comparable to that offered by PET (5–6 mm).[46] In general, SPECT studies have similar utility, are substantially less complex and costly, and are frequently complementary to PET. Some examples of systems for which SPECT has been successfully employed include the use of 3-iodo-6-methoxybenzamide to image the dopamine-D_2 receptor,[47] L-3-iodo-α-methyltyrosine for protein synthesis in brain tumors,[48] Ro-16-0154 for benzodiazepine receptors,[49] and 3(*R*)-quinuclidinyl-4-(*S*)-iodobenzilate for muscarinic receptors,[50] including the first human imaging studies[51] with the latter ligand.

The results of our present study of the epimeric 6β- and 6α-iodo-3,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (1, ioxy and 2, epiioxy) render these ligands potentially useful for SPECT imaging of opioid receptors. SPECT studies with 1 and 2 should compliment our PET program with [^{18}F]cyclofoxy as these compounds have the advantage of being chemically very similar to cyclofoxy and like cyclofoxy show a narcotic-antagonist profile. We also describe an efficient radiochemical synthesis of high specific activity [^{125}I]ioxy ([^{125}I]-1), a ligand that will serve to further assess the utility of [^{123}I]-1 as a ligand suitable for SPECT imaging of human opioid receptors. In addition, the potential of 1 and 2 for such studies is heightened since the unnatural opiate enantiomers of these compounds can be readily prepared by the NIH Opiate Total Synthesis.[21] Appropriate radiolabeling will then provide tools for determination and quantitation of specific binding as was accomplished with cyclofoxy.

Chemistry

The synthetic route to 1 and 2 utilized naltrexone (3)[52] as the starting material (Scheme I). A stereoselective reduction of the carbonyl group of naltrexone was investigated utilizing K-Selectride as the reducing agent. In our previously reported synthesis of 6α-naltrexol (4),[23] we and others[53] used sodium borohydride as the reducing agent

Scheme II.^{a} Synthesis of [^{125}I]Ioxy ([^{125}I]1)



^{a}(a) [^{125}I]NaI, 76 °C, 1.5 h, CH_3CN; (b) concentrated aqueous NH_3 solution/CH_3CN (1:1), 25 °C.

and obtained both epimeric alcohols. Reduction of naltrexone (3) with K-Selectride at 5 °C stereospecifically afforded 6α-naltrexol (4). This was isolated in 75% recrystallized yield as its (*R*)-(−)-mandelate salt. The 6β-naltrexol (5) was obtained in 89% recrystallized yield as described previously[54] using formamidinesulfinic acid as the reducing agent. Careful attention was paid to the reaction temperature to avoid opening of the oxide bridge. Treatment of 4 with acetic anhydride in the presence of excess aqueous sodium hydrogen carbonate as described previously[23] for the synthesis of 3-acetylmorphine[55] afforded the corresponding 3-*O*-acetyl-6α-naltrexol (6) in quantitative yield. 3-*O*-acetyl-6β-naltrexol (7) was also obtained using this route. The corresponding 6α- and 6β-triflate esters 8 and 9 (Scheme I) were obtained in high yield by a modification[30] of our previously reported method[23] for synthesis of 6α-triflate ester 8. Thus, treatment of 6 from −30 to 10 °C with trifluoromethanesulfonic acid anhydride in chloroform in the presence of freshly redistilled *N*-methylmorpholine[30] afforded triflate ester 8 in 81% yield after purification by column chromatography on silica gel. Attempts to crystallize purified 8 were unsuccessful. The 6β-triflate ester 9 was similarly obtained in 92% yield. Interestingly, 9 was highly crystalline, unlike its epimer 8. Treatment of 8 with tetraethylammonium iodide from −10 to 23 °C during 4 h afforded a 90% yield of 6β-iodo-3-acetyl-6-desoxynaltrexone (10) as a crystalline base. Single-crystal X-ray analysis of 10 base confirmed the 6β-configuration of the iodine atom. Additionally, ^{1}H-NMR comparison of the C5-proton of 1 (derived from 10 by deacetylation) [4.97 (d, $J_{5\beta,6\alpha}$ = 8.1 Hz)] with the same proton [4.61 (dd, $J_{5\beta,6\alpha}$ = 6 Hz, $J_{5\beta,6\beta\text{-F}}$ = 21 Hz)] in our previously reported 6β-fluoro derivative (cyclofoxy[19,23,25]) supports the results obtained from X-ray crystallography. Compound 10 was easily separated from its starting triflate precursor 8. A combination of mass spectral and ^{1}H-NMR analysis of the crude reaction mixture indicated the complete absence of any elimination products or starting material 8. This is in contrast to the result observed during the synthesis of cyclofoxy,[19,23] where significant quantities of side products were formed. The lack of side products in this synthesis offers an advantage

(46) Ichihara, T.; Fujiki, Y.; Iwasaki, T.; Matsuda, H.; Hisada, K.; Matsudaira, M.; Yamada, H. High-Spatial-Resolution SPECT with Fan Beam Collimators for Brain Imaging. *J. Nucl. Med.* **1990**, *31*, S869.
(47) Costa, D. C.; Verhoeff, N. P. L. G.; Cullum, I. D.; Ell, P. J.; Syed, G. M. S.; Barrett, J.; Palazidou, E.; Toone, B.; Van Royen, E.; Bobeldijk, M. In vivo Characterization of 3-Iodo-6-methoxybenzamide ^{123}I in Humans. *Eur. J. Nucl. Med.* **1990**, 1–4.
(48) Biersack, H. J.; Coenen, H. H.; Stocklin, G.; Reichmann, K.; Bockisch, A.; Oehr, P.; Kashab, M.; Rollmann, O. Imaging Brain Tumors with L-[^{123}I]iodo-α-methyltyrosine and SPECT. *J. Nucl. Med.* **1989**, *30*, 110–112.
(49) Beer, H.-F.; Blauenstein, P. A.; Hasler, P. H.; Delaloye, B.; Riccabona, G.; Bangerl, I.; Hunkeler, W.; Bonetti, E. P.; Pieri, L.; Richards, J. G.; Schubiger, P. A. In vitro and in vivo Evaluation of Iodine-123-Ro 16-0154: A New Imaging Agent for Spect Investigations of Benzodiazepine Receptors. *J. Nucl. Med.* **1990**, *31*, 1007–1014.
(50) Gibson, R. E.; Schneidau, T. A.; Cohen, V. I.; Sood, V.; Ruch, J.; Melograna, J.; Eckelman, W. C.; Reba, R. C. In Vitro and In Vivo Characteristics of [iodine-125] 3-(R)-Quinuclidinyl-(S)-4-iodobenzilate. *J. Nucl. Med.* **1989**, *30*, 1079–1087.
(51) Eckelman, W. C.; Reba, R. C.; Rzeszotarski, W. J.; Gibson, R. E.; Hill, T.; Holman, B. L.; Budinger, T.; Conklin, J. J.; Eng, R.; Grissom, M. P. External Imaging of Cerebral Muscarinic Acetylcholine Receptors, *Science* **1984**, *223*, 291–293.
(52) Naltrexone base was kindly supplied by Mallinckrodt Medical Narcotics Division, St. Louis, MO 63147.
(53) Brine, G. A.; Prakash, D.; Hart, C. K.; Kotchmar, D. J.; Moreland, C. G.; Carroll, F. I. Ring C Conformation of 6β-Natrexol and 6α-Natrexol. Evidence from Proton and Carbon-13 Nuclear Magnetic Resonance. *J. Org. Chem.* **1976**, *41*, 3445–3448 and references cited therein.
(54) Chatterjie, N.; Inturrisi, C. E.; Dayton, H. B.; Blumberg, H. Stereospecific Synthesis of the 6-Beta-hydroxy Metabolites of Naltrexone and Naloxone. *J. Med. Chem.* **1975**, *18*, 490–492.
(55) Welsh, L. H. O^3-Monoacetylmorphine. *J. Org. Chem.* **1954**, *19*, 1409–1415.

over our previously reported[24] [^{18}F]cyclofoxy synthesis since it translates to a shorter purification time and thus a higher specific radioactivity in the product. Treatment of triflate ester **9** under the same conditions as for the synthesis of **10** resulted in no significant reaction at 25 °C; however, heating of the reaction mixture to 80 °C for 4 h resulted in a 97% yield of **11** with no observable elimination or other side reactions as determined by ^{1}H-NMR and mass spectral analysis. The lower reactivity of **9** toward iodide compared with **8** was expected as a result of greater steric hindrance to iodide approach. Surprisingly, no scrambling of the stereochemistry of **11** in the 6-position was observed in spite of the more energetic conditions required for its formation. Both **10** and **11** were found to be separable by TLC and distinguishable by a variety of spectroscopic techniques. The final step in the synthesis required cleavage of the 3-acetoxy ester group of **10** and **11**. This was achieved by treatment with concentrated aqueous ammonia in THF/MeOH at 25 °C to give ioxy (**1**) and epiioxy (**2**) in quantitative yield. No elimination or substitution reactions were observed during these ester cleavages. On the basis of both considerations of ease of formation as well as potency in vivo and in vitro (see Biological Section later), we decided to synthesize [^{125}I]-**1** and evaluate it for potential to label opioid receptors in vivo.[56] A further consideration in the synthesis of [^{125}I]-**1** as opposed to [^{125}I]-**2** is the structural similarity of [^{125}I]-**1** to the previously reported [^{18}F]cyclofoxy.

Radiochemical Synthesis

Treatment of excess **8** with [^{125}I]sodium iodide (no carrier added) (Scheme II) in acetonitrile at 60 °C for 60 min resulted in a 34.5% radiochemical yield of [^{125}I]-**10** (no carrier added) together with unreacted **8**. A different set of reaction conditions using twice the concentration of precursor, **8** (in 50-μL final volume) a higher reaction temperature (76 °C) and a longer reaction time (90 min) resulted in a quantitative incorporation of carrier-free [^{125}I]-**10**. High-yield conversion of [^{125}I]-**10** to [^{125}I]-**1** was achieved by treatment with 1:1 aqueous ammonia solution/acetonitrile for 30 min at room temperature. As with the unlabeled compounds, no elimination, substitution, 6-iodide atom scrambling, or other side reactions were observed during these radioiodination reactions. The HPLC elution profile (0.9 mL/min isocratic elution with 3:1 aqueous 0.1% trifluoroacetic acid–CH_3CN on a 0.4 × 10 cm 3 μM particle size C18 reverse-phase cartridge column) of the reaction between excess **8** and [^{125}I]sodium iodide is shown in Figure 1. The profile clearly shows one major radiochemical species ([^{125}I]-**10**) (broken line) as well as the excess of unreacted precursor **8** (solid line). Peaks (solid lines) occurring between 0 and 10 min are column/solvent artifacts. The shorter retention time of [^{125}I]-**10** compared with **8** (large excess) proved to be a major advantage for separation of [^{125}I]-**10** from **8**. A small amount (ca. 5%) of deacetylation of [^{125}I]-**10** to [^{125}I]-**1** was observed under the conditions of the reaction (76 °C, 1.5 h, acetonitrile), presumably due to traces of water in the acetonitrile.

X-ray Crystallographic Analysis of 3-Acetoxy-6β-iodo-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (10)

Crystals of **10**, $C_{22}H_{26}NO_4I$, FW = 495.3 were grown by

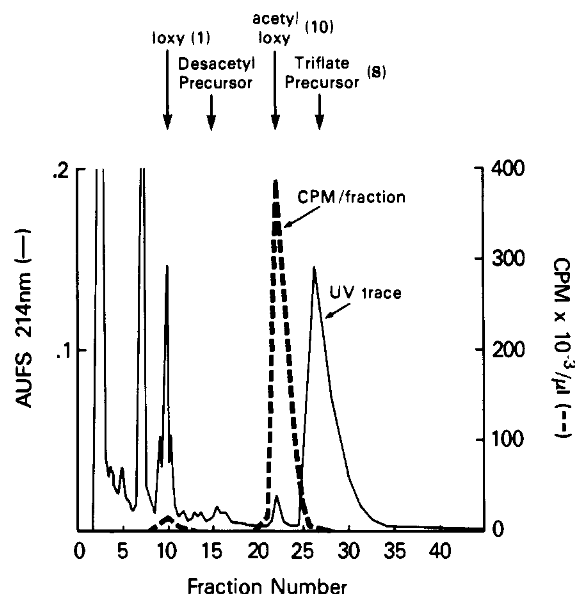


Figure 1. HPLC elution profile (0.9 mL/min isocratic elution with 0.1% aqueous trifluoroacetic acid–CH_3CN 3:1 on a 0.4 × 10 cm, 3-μm particle size C18 reverse-phase cartridge column) of the reaction between excess **8** and [^{125}I]sodium iodide. Broken lines indicate radiochemical profile while solid lines represent the UV absorbance profile measured at 214 nM. Peaks (solid lines) occurring between 0 and 10 min are column/solvent artifacts and should be ignored. Reinjection of the HPLC purified [^{125}I]-**10** indicated one radiochemically pure (>99%) species comigrating with unlabeled **10**.

slow cooling of a solution of **10** in 3:7 ethyl acetate–*n*-hexane. A clear 0.34 × 0.40 × 0.48 mm crystal was selected for data collection. Data were collected on a computer controlled diffractometer with an incident beam graphite monochromator (Nicolet R3m/V with Mo Kα radiation, λ = 0.71073 Å, T = 295 K). A least-squares refinement using 25 centered reflections within 50° < 2θ < 80° gave the triclinic $P1$ cell a = 6.994 (2) Å, b = 8.513 (2) Å, c = 9.660 (2) Å, α = 64.22 (2)°, β = 83.28 (2)°, and γ = 89.06 (2)°, with V = 513.9 (2) Å^3, Z = 1, and d_{calc} = 1.60 g/cm^3. A total of 2954 independent reflections were measured in the $\theta/2\theta$ mode to $2\theta_{max}$ = 55°. Corrections were applied for Lorentz and polarization effects. A semiempirical absorption correction based on the ϕ dependence of 12 reflections with $\chi \approx 90°$ was applied, μ = 1.57 mm^{-1}, and maximum and minimum transmission was 0.92 and 0.75, respectively. The structure was solved by direct methods with the aid of the program SHELXTL[57] and refined with a full matrix least-squares.[57] The 273 parameters refined include the coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Carbon hydrogens were included using a riding model in which the coordinate shifts of the carbon atoms were applied to the attached hydrogen atoms, C–H = 0.96 Å, H angles were idealized, and $U_{iso}(H) = 1.2U_{eq}(C)$, except for those on the cyclopropylmethyl group and the hydroxyl hydrogen, which were refined isotropically. The final R value for the 2811 (includes ca. 200 Friedel pairs) observed reflections with $F_o > 3\sigma(|F_o|)$ were R = 0.027, and wR = 0.035, where $w = 1/[\sigma^2(|F_o|) + g(F_o)^2]$ and g = 0.00023. The goodness of fit parameter was 1.71 and final difference Fourier excursions were 0.30 and −0.97 eÅ^{-3}.

The absolute configuration determination was based on the method suggested by D. Rogers.[58] The parameter η

(56) Seeger, T. S.; Sforzo, G. A.; Pert, C. B.; Pert, A. In Vivo Autoradiography: Visualization of Stress-induced Changes in Opiate Receptor Occupancy in the Rat Brain. *Brain Res.* 1984, *305*, 303–311. The results of in vivo labeling with [^{125}I]-1 will be presented elsewhere.

(57) Sheldrick, G. M. SHELXTL80. *Minicomputer Programs for Structure Determination*, University of Gottingen, Sweden, 1980.

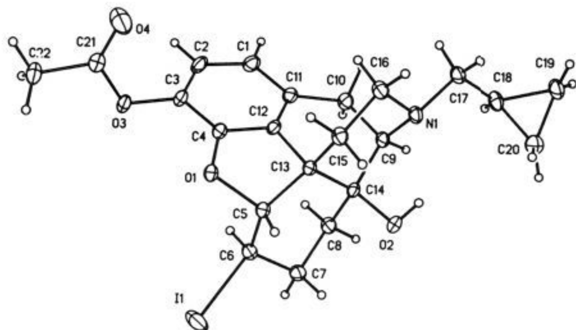


Figure 2. Thermal ellipsoid plot drawn from experimental coordinates of 3-acetoxy-6β-iodo-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (**10**). Thermal ellipsoids are drawn at the 20% probability level.

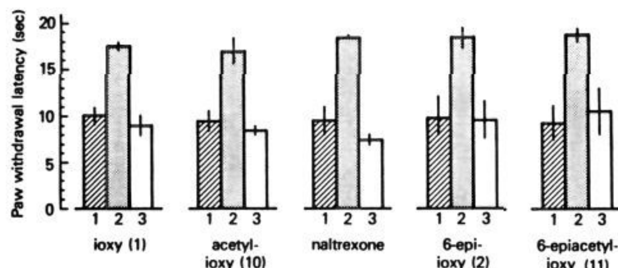


Figure 3. Reversal of morphine analgesia. (1) 0.1 mL normal saline/100 g of body weight, subcutaneous; (2) 10 mg/kg morphine sulfate in a volume of 0.1 mL normal saline/100 g of body weight, subcutaneous; (3) naltrexone, **1**, **2**, **10**, **11** (5 mg/kg each in 0.1 mL normal saline/100 g of body weight), intravenously, 40 min after the morphine administration.

which multiplies all $\Delta F''$ values (imaginary component of atomic scattering factor) refines to a value of $\eta = 1.03$ (4). A correct choice of enantiomer would give +1.0 and an incorrect choice −1. In addition, the wR for the choice of the other enantiomer is 0.049, significantly above that of the correct hand.

In Figure 2, the asymmetric carbons are 5*R*, 6*R*, 9*R*, 13*S*, and 14*S*. The 6*R* configuration in ring C, which is in a chair conformation, results in the iodine substituent being equatorial to the ring. Orientation of the acetoxy and cyclopropylmethyl groups with respect to the fused ring moiety may be described by the torsion angles C2–C3–O3–C21 = −80.7° and C9–N1–C17–C18 = −57.4°, respectively. Bond distances and angles are within normal ranges, and tables of coordinates, bond distances and angles, and anisotropic thermal parameters are available as supplementary material. The only intermolecular contact shorter than 3.55 Å for O···I van der Waals contact occurs for O4··I1[x, 1.0 + y,z] = 3.34 Å.

Results and Discussion

Ioxy (**1**) and epiioxy (**2**) were evaluated in rat and guinea pig brain membranes for their opiate receptor subtype selectivity and potency (Table I). The antagonist properties of **1** and **2** and acetate esters **10** and **11** were evaluated in vivo in rats (Figure 3). These in vivo studies used the rat paw withdrawal latency test and indicated that all of the compounds (**1**, **2**, **10**, and **11**), like naltrexone, could produce a complete reversal of the effects of morphine. The results of this in vivo study also indicated that the compounds were effective in penetration of the blood–brain barrier, an important consideration in the development of SPECT or PET scanning ligands for brain receptor imaging. The results also showed the 3-*O*-acetates **10** and **11** to be equiactive within the paw withdrawal test (Figure 3), presumably due to enzymatic 3-*O*-acetoxyl hydrolysis analogous to the rapid conversion of heroin to 6-*O*-acetylmorphine.[59]

In vitro in the rat (Table I) against [^{3}H]cyclofoxy (a ligand that generally labels μ-[60,61] and κ_2-receptor bind-

Table I. Opiate Receptor Subtype Selectivity of Iodinated Opiates[a]

	IC_{50} (nM)	K_i (nM)
μ- and κ_2-Receptor Binding; [^{3}H]Cyclofoxy (K_d = 0.8 nM; ligand concentration = 1.3 nM)		
ioxy	0.77 ± 0.05	0.29
epiioxy	4.34 ± 0.19	1.65
cyclofoxy	8.98 ± 0.35	3.42
naltrexone	6.77 ± 0.18	2.57
cyclobroxy	3.14 ± 0.11	1.19
High-Affinity δ-Receptors; [^{3}H]DADLE (K_d = 1.6 nM; ligand concentration = 1.9 nM)		
ioxy	25.6 ± 3.1	11.7
epiioxy	101 ± 8.4	46.2
cyclofoxy	268 ± 33	122
naltrexone	221 ± 31	101
cyclobroxy	4.30 ± 0.46	1.96
Low-Affinity δ-Receptors; [^{3}H]DADLE (K_d = 12.2 nM; ligand concentration = 2.1 nM)		
ioxy	2.64 ± 0.21	2.25
epiioxy	8.06 ± 0.58	6.88
cyclofoxy	16.2 ± 0.8	13.8
naltrexone	5.57 ± 0.31	4.75
cyclobroxy	5.77 ± 0.14	4.92
κ_2-Receptor Binding; [^{3}H]BRM (K_d = 1.0 nM; ligand concentration = 1.8 nM)		
ioxy	7.65 ± 0.23	2.73
epiioxy	23.7 ± 1.1	8.46
cyclofoxy	66.0 ± 3.9	23.5
naltrexone	47.2 ± 1.9	16.8
cyclobroxy	20.2 ± 1.2	7.21
κ_1-Receptor Binding; [^{3}H]U69,593 (K_d = 1.6 nM; ligand concentration = 1.8 nM)		
ioxy	0.89 ± 0.01	0.42
epiioxy	3.17 ± 0.06	1.49
cyclofoxy	7.88 ± 0.09	3.71
naltrexone	5.97 ± 0.24	2.81
cyclobroxy	0.70 ± 0.02	0.32
μ-Receptor Binding; [^{3}H]DAGO (K_d = 0.7 nM; ligand concentration = 1.7 nM)		
ioxy	2.74 ± 0.21	0.80
epiioxy	7.16 ± 0.72	2.09
cyclofoxy	11.4 ± 0.05	3.32
naltrexone	4.04 ± 0.15	1.18
cyclobroxy	4.21 ± 0.14	1.23

[a] Each [^{3}H]ligand was displaced by eight concentrations of test drug. The data of two experiments were combined and fit to the two parameter logistic equation[70] for the best-fit estimates of the IC_{50} and the slope factor. The K_d values of the respective ligands were as follows: [^{3}H]DAGO (0.7 nM), [^{3}H][D-Ala^2,D-Leu^5]enkephalin (1.6 nM at the δ_{ncx} site, 12.2 nM at the δ_{cx} site), [^{3}H]U69,593 (1.6 nM), [^{3}H]bremazocine (1.0 nM), [^{3}H]cyclofoxy (0.8 nM).

(58) Rogers, D. On the Application of Hamilton's Ratio Test to the Assignment of Absolute Configuration and an Alternative Test. *Acta Crystallogr.* **1981**, *A37*, 734–741.

(59) Inturrisi, C. E.; Max, M. B.; Foley, K. M.; Schultz, M.; Shin, S-U.; Houde, R. W. The Pharmacokinetics of Heroin in Patients with Chronic Pain. *N. Engl. J. Med.* **1984**, *310*, 1213–1217.

(60) Rothman, R. B.; Bykov, V.; Reid, A.; de Costa, B. R.; Hauck-Newman, A.; Jacobson, A. E.; Rice, K. C. A Brief Study of the Selectivity of Norbinaltorphimine, (−)-Cyclofoxy and (+)-Cyclofoxy Among Opioid Receptors In Vitro. *Neuropeptides* **1988**, *12*, 181–187.

ing[18]), ioxy (1) exhibited a K_i of 0.29 nM. However its epimer (2) exhibited a K_i of 1.65 nM or a 6-fold reduction in affinity, indicating that the 6β-configuration is more favorable than the 6α-configuration in this assay. Cyclofoxy containing the smaller 6β-fluorine atom exhibited a 12-fold lower affinity compared with 1, indicating that the larger iodine atom is beneficial for its opioid receptor affinity. This is further exemplified with the 6β-bromo analog[30] of 1 (cyclobroxy), which shows an intermediate receptor affinity (1.19 nM). Interestingly, ioxy was also more potent than the opiate antagonist, naltrexone (K_i = 2.57 nM) in this assay.

Similar relative in vitro potency as that seen with displacement of [^{3}H]cyclofoxy was observed for these compounds (except cyclobroxy) at the high-affinity δ-site ([^{3}H]DADLE)[62] in the rat (Table I). Thus, ioxy (1) exhibited an affinity of 11.7 nM while epiioxy exhibited a 4-fold lowered affinity (K_i = 46.2 nM). As in the displacement of [^{3}H]cyclofoxy, cyclofoxy was also approximately 10-fold less potent than ioxy for displacement of [^{3}H]DADLE from the high-affinity δ-site. Naltrexone exhibited comparable affinity while cyclobroxy displaced [^{3}H]DADLE with a 6-fold higher affinity than ioxy.

For displacement of [^{3}H]DADLE from the low-affinity δ-site,[62] ioxy exhibited the highest affinity of all the compounds reported in Table I. Epiioxy showed a 3-fold lower affinity (K_i = 6.88 nM) and cyclofoxy showed a 6-fold lower affinity, again corroborating the beneficial effect of the larger iodine atom and 6β-configuration on opioid receptor binding as seen above. Both naltrexone and cyclobroxy were intermediate or roughly half as potent as ioxy at this site.

In guinea pig membranes pretreated with the site-directed affinity ligands[18,63] 2-(4-ethoxybenzyl)-1-[2-(diethylamino)ethyl]-5-isothiocyanatobenzimidazole (BIT)[64] and *N*-phenyl-*N*-[1-[2-(4-isothiocyanato)phenethyl]-4-piperidinyl]propanamide (FIT),[64] to irreversibly deplete μ- and δ-sites, respectively, the displacement of the nonselective opioid [^{3}H]bremazocine ([^{3}H]BRM) is a measure of κ_2-receptor binding.[18,63] Thus (Table I), ioxy displaced [^{3}H]BRM with a K_i of 2.73 nM while epiioxy (2) was 3-fold less potent in this respect. Cyclofoxy containing the smaller F atom was less potent by a factor of 7–9-fold. Naltrexone was 7-fold less potent than ioxy, and cyclobroxy was 3-fold less potent.

[^{3}H]U69,593 displacement from guinea pig membranes pretreated with the site-directed affinity ligands BIT[64] and FIT[64] (to deplete μ- and δ-receptors, respectively) is a good measure of κ_1-receptor binding affinity.[18] Of the compounds tested (Table I), ioxy (K_i = 0.42 nM) and cyclobroxy (K_i = 0.32 nM) were the most potent displacers of [^{3}H]U69,593 under these conditions. Epiioxy (2) was 4-fold less potent while cyclofoxy was 9-fold less potent (as it was for κ_2-receptors). Similarly, naltrexone was 7-fold less potent.

Displacement of [^{3}H]DAGO (Table I) from rat brain membranes is a versatile measure of μ-receptor binding affinity.[65] Among all of the compounds tested at this receptor, ioxy was the most potent. Its epimer (2) showed a 2-fold lower affinity, and cyclofoxy was 4-fold less potent. Naltrexone and cyclobroxy were both about 1.5-fold less potent at this site.

On the basis of both in vivo (Figure 3) and in vitro (Table I) opioid receptor potency, we selected ioxy instead of epiioxy for radioiodination. In the radioiodination experiments, the tetraethylammonium iodide that was used in the unlabeled work was substituted with sodium ^{125}-iodide. The conditions employed utilized anhydrous carrier-free Na^{125}I in dry acetonitrile. Optimization of the conditions (76 °C for 1.5 h) resulted in a quantitative yield of [^{125}I]-10. As with unlabeled 10, deprotection of the 3-*O*-acetyl group occurred smoothly in the presence of excess concentrated aqueous ammonia/acetonitrile to give the desired [^{125}I]-1 in 88.5% radiochemical yield after HPLC purification on an analytical-scale reverse-phase (C18) cartridge column. During radiochemical synthesis, it was found to be important to completely separate [^{125}I]-10 from excess unreacted precursor 8 since failure to do so resulted in contamination of the [^{125}I]-1. Preliminary binding experiments indicated that unlabeled 1 could displace [^{125}I]-1 with a K_d of 0.18 nM.[66] Preliminary in vivo labeling experiments using both [^{125}I]-1 and [^{125}I]-10 indicated that they could label opiate-rich areas of rat brain as determined by autoradiography and in living rats by use of a "γ-camera".[67]

Conclusion

The in vivo experiments proved ioxy to be a potent opioid receptor antagonist in the rat. The experiments demonstrated that it readily passed the blood–brain barrier. In general, ioxy was more potent in vitro than the other compounds tested in Table I at all of the opioid receptor subtypes. A qualitative examination of atom type in the 6-position versus receptor potency indicated that I > Br > F. Ioxy exhibited a greater degree of κ selectivity (κ_1/μ = 1.9) (κ_2/μ = 0.3) than cyclofoxy ($\kappa_{1/\mu}$ = 0.89) (κ_2/μ = 0.14).

A combination of both receptor binding data and in vivo potency (after iv administration) of 1 and 10 together with preliminary in vivo receptor localization experiments with [^{125}I]-1 and [^{125}I]-10[67] strongly indicate that the ^{123}I-labeled versions of these compounds will be suitable for SPECT labeling of opioid receptors in living subjects. This is further supported by the close structural similarity of 1 and 10 to cyclofoxy, which our animal[19,24,27,32-35] and human[27,29,31,41] studies have shown to be an excellent imaging agent for opioid receptors in vivo.

Experimental Section

Biological Materials and Methods. In Vivo Antagonist Activity. Assessment of the ability of this series of drugs to cross the blood–brain barrier was tested in male Sprague–Dawley rats (300 g) acutely treated with morphine (see Figure 3). We expected

(61) Rothman, R. B.; McLean, S. An Examination of the Opiate Receptor Subtypes Labeled by [^{3}H]cyclofoxy: An Opiate Antagonist Suitable for Positron Emission Tomography. *Biol. Psych.* 1988, *23*, 435–458.
(62) Rothman, R. B.; Bykov, V.; Ofri, D.; Rice, K. C. LY164929: A Highly Selective Ligand for the Lower Affinity [^{3}H][D-Ala^2,D-Leu^5]enkephalin Binding Site. *Neuropeptides* 1988, *11*, 13–16.
(63) Rothman, R. B.; Bykov, V.; Danks, J. A.; Jacobson, A. E.; Burke, T. R., Jr.; Rice, K. C.; Herkenham, M. Preparation of Rat Brain Membranes Highly Enriched with Opiate Kappa Binding Sites Using Site-Directed Acylating Agents: Optimization of Assay Conditions. *Neuropeptides* 1985, *6*, 503–515.
(64) Rice, K. C.; Jacobson, A. E.; Burke, T. R., Jr.; Bajwa, B. S.; Streaty, R. A.; Klee, W. A. Irreversible Ligands with High Selectivity Toward Delta or Mu Opiate Receptor Subtypes. *Science* 1983, *220*, 314–316.
(65) (a) Rothman, R. B.; Long, J. B.; Bykov, V.; Jacobson, A. E.; Rice, K. C.; Holaday, J. W. Beta-FNA Binds Irreversibly to the Opiate Receptor Complex. In vivo and In vitro Evidence. *J. Pharmacol. Exp. Ther.* 1988, *247*, 405–416. (b) Kosterlitz, H. W.; Patterson, S. J.; Tyr-D-ala^2-gly-me-phe-NH(CH$_2$)$_2$-OH is a selective ligand for the mu opioid binding site. *Br. J. Pharmacol.* 1981, *73*, 299P.
(66) Rothman, R. B.; et al. Unpublished results.
(67) Iadarola, M.; Berman, K.; et al. Unpublished results.

that the drugs would have antagonist activity and determined whether they could reverse morphine-induced analgesia. A baseline paw withdrawal to a radiant thermal stimulus was obtained for unrestrained animals as described previously;[68,69] the stimulus was set to give a baseline withdrawal latency of approximately 10 s and the cutoff was at 18 s. After baseline testing, morphine sulfate, 10 mg/kg, was injected subcutaneously in a volume of 0.1 mL of saline/100 g of body weight. By 40 min, the rats were fully analgesic; most reached the 18 s cutoff and showed characteristic opioid-induced behavioral effects. Naltrexone and the 3-O-acetylated (10 and 11) and deacetylated (1 and 2) ioxy epimers were administered intravenously (5 mg/kg each, in 0.1 mL saline/100 g of body weight). The opioid effects were reversed in a matter of seconds by all of the ioxy derivatives and naltrexone. Withdrawal latency, tested within 5–10 min of the iv injection, returned to near baseline values in all cases. While not shown in the figure, the reversal lasted for at least 40 min.

Receptor Binding. μ-Binding sites were labeled using 1.7 nM [^{3}H]DAGO (SA = 40.8 Ci/mmol) and rat lysed P2 membranes as previously described.[65] Briefly, incubations proceeded for 4–6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing a protease inhibitor cocktail [bacitracin (100 μg/mL), bestatin (10 μg/mL), leupeptin (4 μg/mL), and chymostatin (2 μg/mL)]. Nonspecific binding was determined using 20 μM levallorphan. Higher affinity (δ_{ncx}) and lower affinity (δ_{cx}) δ-binding sites were labeled using 1.9 nM [^{3}H][D-Ala^2,D-Leu^5]enkephalin (SA = 30 Ci/mmol) and rat lysed P2 membranes as previously described.[62] Briefly, incubations proceeded for 4–6 h at 25 °C in 10 mM Tris-HCl, pH 7.4, containing 100 mM choline chloride, 3 mM $MnCl_2$, and the protease inhibitor cocktail. 100 nM MeTyr-D-Ala-Gly-N(Et)-$CH(CH_2Ph)CH_2N(CH_3)_2$ (LY164929) was used to block binding to the δ_{cx} binding site, and 100 nM [D-pen^2,L-pen^5]enkephalin was used to block binding to the δ_{ncx} binding site. Nonspecific binding was determined using 20 μM levallorphan. [^{3}H]Cyclofoxy binding sites (μ plus κ_2) were labeled using 1.3 nM [^{3}H]cyclofoxy (SA = 20.6 Ci/mmol) and rat brain lysed P2 membranes as previously described.[61] Nonspecific binding was determined using 20 μM levallorphan. κ_1-Binding sites were labeled using 1.8 nM [^{3}H]-U69,593 (SA = 40 Ci/mmol) and guinea pig brain membranes depleted of μ- and δ-binding sites by pretreatment with BIT and FIT as previously described,[18] except that the incubation temperature was 25 °C. Briefly, incubations proceeded for 4–6 h at 25 °C in 50 mM Tris-HCl, pH 7.4, containing the protease inhibitor cocktail plus 1 μg/mL captopril. Nonspecific binding was determined using 1 μM U69,593. κ_2-Binding sites were labeled with 1.8 nM [^{3}H]bremazocine using guinea pig brain membranes depleted of μ- and δ-binding sites by pretreatment with BIT and FIT as previously described.[18] Briefly, incubations proceeded for 4–6 h at 0 °C in 50 mM potassium phosphate buffer, pH 7.4, with the same protease inhibitor cocktail used for the [^{3}H]U69,593 binding assay. Nonspecific binding was determined with 1 μM (–)-bremazocine.

Synthesis. Melting points were determined on a Thomas-Hoover capillary apparatus and are uncorrected. Specific rotation determinations at the sodium D line were obtained in a 1 dm cell using a Perkin-Elmer 241-MC polarimeter (automatic). Gas chromatographic (GC) analysis was performed on a Hewlett-Packard 5880A instrument fitted with a 30 M SE-30 capillary column and a flame ionization detector. Elemental analyses were performed at Atlantic Microlabs, Atlanta, GA. Where compounds are indicated by molecular formulae followed by the symbols of the elements (C, H, N), elemental compositions were determined to be within ±0.4% of the calculated values. Chemical-ionization mass spectra (CIMS) were obtained using a Finnigan 1015 mass spectrometer. Electron ionization mass spectra (EIMS) and high-resolution mass measurements (HRMS) were obtained using a VG-Micro Mass 7070F mass spectrometer. ^{1}H-NMR spectra were obtained in $CDCl_3$ solutions using a Varian XL-300 spectrometer. Infrared (IR) spectra were determined using a Bio-Rad FTS-45 FTIR spectrometer. Thin-layer chromatography (TLC) was performed on 250-μm Analtech GHLF silica gel plates. TLC system A corresponds to $CHCl_3$–MeOH–concentrated aqueous NH_3 (90:9:1). TLC system B corresponds to $CHCl_3$–MeOH–concentrated aqueous NH_3 (99:0.9:0.1). TLC system C corresponds to $CHCl_3$–MeOH–concentrated aqueous NH_3 (98:1.8:0.2). All reaction steps involving the use of ^{125}I were performed using carrier-free iodine. The reaction conditions were determined using unlabeled precursors and the products confirmed spectroscopically prior to use of the ^{125}I. Radioactivity measurements were determined using a radioisotope calibrator, Model CRC-10 (Capintec Inc., Montvale, NJ).

6α-Naltrexol (3,6α,14-Trihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan) (4). To a stirred solution of naltrexone base (21.6 g, 63.3 mmol) in dry THF (500 mL), at ambient temperature under argon, was added 14.5 mL of a 35% suspension of KH in mineral oil (126.6 mmol) and stirring was continued until the vigorous effervescence had subsided. The stirred solution was cooled to 5 °C, treated dropwise during 25 min with 95 mL (95 mmol) of a 1.0 M solution of K-Selectride in THF, and stirred for 40 min at 5 °C and then for 12 h at 25 °C. The reaction was quenched by addition of 40 mL of water and the solvent was evaporated in vacuo. To the residue was added 400 mL of water and the aqueous mixture treated with concentrated aqueous HCl to pH 3–4. The acidic solution was extracted with ether (3 × 200 mL) and the ether extract discarded. Treatment of the aqueous layer with excess aqueous ammonia precipitated the free base. Extraction of the aqueous mixture with CH_2Cl_2 (3 × 100 mL), drying of the extract by passage through a short column of Na_2SO_4, and evaporation of the solvent in vacuo gave the crude product 21.7 g (quantitative) as a foam. Analysis of the mixture by TLC (system A) and ^{1}H NMR indicated the absence of epimeric (6β-) alcohol 5 in the reaction product. A small portion of the crude product was crystallized from acetonitrile to give base slightly contaminated with unreacted naltrexone: $[\alpha]_D$ = –202° (c 0.735, $CHCl_3$). The total crude product (base) was dissolved in 150 mL of 2-propanol at 60 °C and treated with 9.63 g (63.3 mmol) of (R)-(–)-mandelic acid. Crystallization occurred spontaneously on cooling to 25 °C. The crystals were filtered and washed with 3 × 20 mL of cold (4 °C) 2-propanol followed by ether (20 mL) and dried in vacuo at 60 °C to afford 4·(R)-(–)-mandelate (23.6 g, 75%) which was free of unreacted naltrexone (TLC, ^{1}H NMR); mp 163–165 °C. Anal. ($C_{28}H_{33}NO_7$·0.75 H_2O) C, H, N.

4 (base): To a mixture of 4·(R)-(–)-mandelate (22.58 g, 46.89 mmol), distilled water (200 mL), and $CHCl_3$ (200 mL) was added 1.87 g (46.89 mmol) of NaOH pellets or standardized 1.0 M aqueous NaOH solution, and the mixture stirred for 10 min at ambient temperature. The organic layer was separated and the aqueous layer was washed with 3 × 100 mL of $CHCl_3$. The combined organic layer was dried (Na_2SO_4) and evaporated to give 4 (base) (quantitative) as a colorless foam. Crystallization from cold (5 °C) acetonitrile (100 mL) afforded 10.62 g of pure 4. Evaporation of the acetonitrile filtrate to 50 mL afforded a further 4.70 g of pure product: mp 208–209 °C; ^{1}H NMR ($CDCl_3$) δ 6.71 (d, J = 6.2 Hz, 1 H), 6.52 (d, J = 6.2 Hz, 1 H), 4.65 (d, J = 4.3 Hz, 1 H), 4.24 (m, 1 H) (identical to that previously described[23]); IR ($CHCl_3$) 3581, 3364, 3008, 2938, 2832, 1508, 1460, 1391, 1336, 1148, 1084, 976 cm^{-1}; $[\alpha]_D$ = –214° (c 0.96, $CHCl_3$). Anal. ($C_{20}H_{25}NO_4$) C, H, N.

6β-Naltrexol (3,6β,14-Trihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan) (5). To a suspension of naltrexone base (6.81 g, 20.0 mmol) under argon was added 100 mL (enough to afford complete solution) of 0.533 M aqueous NaOH. The alkaline solution of naltrexone was treated dropwise at ambient temperature during 20 min with 8.64 g (80 mmol) of formamidinesulfinic acid dissolved in 200 mL of 0.533 M aqueous NaOH. After the addition was complete, the solution was heated and stirred at 80–85 °C for 1.5 h when TLC (system A) indicated the reaction to be complete. The reaction mixture was cooled (ice bath) and then treated dropwise under argon with a solution of ammonium chloride (10.27 g, 192 mmol) in distilled water (100

(68) Iadarola, M. J.; Douglass, J.; Civelli, O.; Naranjo, J. R. Differential Activation of Spinal Cord Dynorphin and Enkephalin Neurons During Hyperalgesia: Evidence Using cDNA Hybridization. *Brain Res.* **1988**, *455*, 205–212.
(69) Hargreaves, K.; Dubner, R.; Brown, F.; Flores, C.; Joris, J. A New and Sensitive Method for Measuring Thermal Nociception in Cutaneous Hyperalgesia. *Pain* **1988**, *32*, 77–88.
(70) Rodbard, D.; Lenox, R. H.; Linton, Ramseth, D. Statistical Characterization of the Random Errors in the Radioimmunoassay Dose–response Variable. *Clin. Chem.* **1976**, *22*, 350–8.

mL). The aqueous mixture was extracted with 5 × 100 mL of $CHCl_3$ and the combined organic extract was filtered through a pad of Na_2SO_4 and evaporated in vacuo to afford crude **5** (base) as a foam which was dissolved in 20 mL of warm (50 °C) ethyl acetate and diluted to 60 mL with warm *n*-hexanes. Crystallization occurred spontaneously on cooling. The crystals were collected by filtration, washed with 2 × 10 mL of cold ethyl acetate–*n*-hexanes (1:3), and oven-dried in vacuo at 60 °C to give **5** (6.11 g, 89%) (free of any 6α-epimer as determined by ^1H NMR and TLC): mp 187–188 °C, (lit.[54] mp 188–190 °C); ^1H NMR ($CDCl_3$) δ 6.71 (d, J = 8.1 Hz, 1 H), 6.56 (d, J = 8.1 Hz, 1 H), 4.55 (d, J = 6.1 Hz, 1 H), 3.57 (m, 1 H) (identical to that previously described[54]); IR ($CHCl_3$) 3587, 3356, 3009, 2950, 2833, 1506, 1455, 1325, 1093, 1034, 978 cm^{-1}; $[\alpha]_D$ = −156° (*c* 0.604, MeOH) [lit.[54] $[\alpha]_D$ = −133.8° for **5**·HCl (*c* 1, H_2O)].

3-Acetoxy-6β,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (7). The method of Welsh[55] for acetylation of morphine to morphine 3-acetate as applied earlier[23] was altered as follows. Compound **5** (base) (5.50 g, 16.0 mmol) was suspended in 180 mL of distilled water in a large beaker, and to this was added 22.9 g (272.6 mmol) of $NaHCO_3$. To the vigorously stirred mixture was cautiously added dropwise 13.7 mL of acetic anhydride. Voluminous effervescence and foaming occurred during the addition, and after 20 min, the reaction had subsided and a clear solution remained. The aqueous mixture was extracted with $CHCl_3$ (5 × 100 mL) and the organic extract was dried through a column of Na_2SO_4, and evaporated in vacuo to afford **7** (base) (quantitative) as an oil (homogeneous on TLC, solvent system A) which failed to crystallize as a number of different salts: ^1H NMR ($CDCl_3$) δ 6.81 (d, J = 8.2 Hz, 1 H), 6.65 (d, J = 8.2 Hz, 1 H), 4.52 (d, J = 5.7 Hz, 1 H), 3.58 (m, 1 H), 3.03–3.13 (m, 2 H), 2.67 (m, 2 H), 2.36 (d, J = 6.6 Hz, 2 H), 2.30 (s, 3 H), 2.12–2.29 (complex m, 3 H), 1.93 (td, J = 8.9, 1.7 Hz, 1 H), 1.49–1.69 (complex m, 3 H), 1.36 (m, 1 H), 0.84 (m, 1 H), 0.55 (m, 2 H), 0.12 (m, 2 H); IR ($CHCl_3$) 3600, 3380, 3080, 3010, 2960, 2840, 1760, 1620, 1490, 1455, 1370, 1200, 1090, 1040, 980 cm^{-1}; HRMS $C_{22}H_{27}NO_5$ requires 385.1889, M^+ (found) 385.1877.

3-Acetoxy-6α,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (6). Compound **4** (base) (12.00 g, 35.2 mmol) was treated with 50 g (595 mmol) of $NaHCO_3$ and 30 mL (317 mmol) of acetic anhydride[23,55] as above for **7** to afford **6** (base) (13.5 g, quantitative) as a colorless oil. Compound **6** (13.09 g) was treated with 3.09 g of oxalic acid in 100 mL of 1:1 acetone–2-propanol. After addition of the oxalic acid, copious crystallization occurred. The suspension of crystals was cooled to 4 °C and then filtered and washed twice with acetone–2-propanol (1:1) to afford 12.97 g of **6**·oxalate: mp 186–187 °C (lit.[23] mp 184–187 °C); ^1H NMR ($CDCl_3$) δ 4.62 (d, J = 5.1 Hz, 1 H) (identical to that previously described[23]).

3-Acetoxy-6α-[[(trifluoromethyl)sulfonyl]oxy]-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (8). The following modification provided more satisfactory results than that reported earlier.[23] To a solution of **6** (base) (11.65 g, 30.3 mmol) in 300 mL of alcohol-free dry chloroform under argon was added 13.3 mL (121 mmol) of freshly redistilled *N*-methylmorpholine, and the solution was cooled to −30 °C. To the cooled and stirred solution was added dropwise, at such a rate that the temperature of the solution did not rise above −30 °C, trifluoromethanesulfonic acid anhydride (10.2 mL, 60.5 mmol). The solution was stirred from −30 to −10 °C during 1 h and then at 0 °C for 10 min when TLC (system B) indicated complete reaction. The reaction mixture was diluted with 300 mL of $CHCl_3$ and washed with 3 × 300 mL of saturated $NaHCO_3$ followed by 3 × 300 mL of water. Evaporation of the solvent afforded the crude product as an oil which was purified by flash column chromatography on silica gel eluting with solvent system B to afford 12.75 g (81%) of **8** as a colorless gum which was stored at −70 °C: ^1H NMR ($CDCl_3$) δ 6.88 (d, J = 8.2 Hz, 1 H), 6.67 (d, J = 8.2 Hz, 1 H), 5.42 (q, J_{app} = 4.5 Hz, 1 H), 4.76 (d, J = 4.6 Hz, 1 H), 3.13 (m, 1 H), 3.10 (d, J = 18 Hz, 1 H), 2.59–2.69 (m, 2 H), 2.36 (m, 2 H), 2.30 (s, 3 H), 2.23 (m, 2 H), 2.02 (dd, J = 3.6, 9.3 Hz, 1 H), 1.46–1.76 (complex m, 4 H), 0.84 (m, 1 H), 0.55 (m, 2 H), 0.13 (m, 2 H) (identical to that reported previously[23]); IR ($CHCl_3$) 3377, 3028, 2929, 2833, 1762, 1619, 1493, 1452, 1412, 1372, 1196, 939 cm^{-1}; CIMS $C_{23}H_{26}F_3NO_7S$ requires 518 (MH^+), MH^+ found 518; $[\alpha]_D$ = −131° (*c* 1.405, $CHCl_3$).

3-Acetoxy-6β-[[(trifluoromethyl)sulfonyl]oxy]-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (9). To a stirred solution of **7** (base) (6.16 g, 16.0 mmol) and freshly redistilled *N*-methylmorpholine (7 mL, 64 mmol) in dry, alcohol free $CHCl_3$ at −30 °C was added trifluoromethanesulfonic acid anhydride (5.4 mL, 32 mmol) at such a rate that the temperature of the solution did not rise above −30 °C. The solution was stirred from −30 to −20 °C for 10 min when TLC (system C) indicated complete reaction. The reaction mixture was diluted with 100 mL of $CHCl_3$ and washed with 3 × 150 mL of saturated $NaHCO_3$ and 3 × 150 mL of water, and the solvent evaporated in vacuo to afford the crude product as a dark oil. The crude product was purified by flash column chromatography on silica gel eluting with solvent system C, to afford pure **9** (7.60 g, 92%) as a colorless gum. This could be crystallized from a mixture of ethyl acetate (5 mL) and hexanes (30 mL) at 4 °C: mp 151–152 °C; ^1H NMR ($CDCl_3$) δ 6.88 (d, J = 8.2 Hz, 1 H), 6.72 (d, J = 8.2 Hz, 1 H), 4.70 (d, J = 6.6 Hz, 1 H), 4.62 (q, J_{app} = 6.1 Hz, 1 H), 3.03–3.18 (m, 2 H), 2.56–2.72 (m, 2 H), 2.30–2.44 (m, 1 H), 2.38 (d, J = 6.5 Hz, 2 H), 2.28 (s, 3 H), 2.22–2.50 (m, 1 H), 2.01 (td, J = 12.1, 3.5 Hz, 1 H), 2.00 (m, 1 H), 1.72 (dt, J = 13.7, 3.3 Hz, 1 H), 1.53 (dd, J = 12.5, 2.3 Hz, 1 H), 1.42 (td, J = 13.6, 2.8 Hz, 1 H), 0.84 (m, 1 H), 0.55 (m, 2 H), 0.13 (m, 2 H); IR ($CHCl_3$) 3023, 3007, 1763, 1412, 1144, 1039, 989, 520 cm^{-1}; CIMS $C_{23}H_{26}F_3NO_7S$ requires 518 (MH^+), MH^+ found 518; $[\alpha]_D$ = −128° (*c* 1.262, $CHCl_3$). Anal. ($C_{23}H_{26}F_3NO_7S$) C, H, N.

3-Acetoxy-6β-iodo-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (10). To a stirred solution of **8** (base) (9.16 g, 17.7 mmol) in dry acetonitrile (300 mL) at −10 °C under argon was added (in one portion), tetraethylammonium iodide (9.11 g, 35.4 mmol) and the solution stirred at −10 °C for 1 h, and then at 25 °C for 3 h when TLC (system C) indicated the reaction to be complete. The solvent was evaporated at ambient temperature in vacuo, and the colorless residue was dissolved in 500 mL of $CHCl_3$ and washed with water (4 × 100 mL). Evaporation of the solvent afforded **10** (base) (7.90 g, 90%) as a crystalline solid. The residue was dissolved in 25 mL of warm ethyl acetate and the solution was diluted by the addition of 60 mL of warm *n*-hexane. Crystallization occurred spontaneously as the solution cooled. When the mixture had reached ambient temperature, further crystallization was achieved by cooling at 4 °C for 2 h. The crystals were filtered and washed with cold (0 °C) solvent to yield 6.00 g (68%): mp 157.5–158 °C; ^1H NMR ($CDCl_3$) δ 6.83 (d, J = 8.2 Hz, 1 H), 6.68 (d, J = 8.2 Hz, 1 H), 4.97 (d, J = 8.2 Hz, 1 H), 3.87 (m, 1 H), 3.01–3.11 (m, 2 H), 2.60–2.70 (m, 2 H), 2.51–2.60 (m, 2 H), 2.36 (d, J = 6.6 Hz, 2 H), 2.32 (s, 3 H), 2.25 (dd, J = 4.8, 12.4 Hz, 1 H), 2.03–2.16 (m, 2 H), 1.56 (m, 1 H), 1.41–1.50 (m, 2 H), 1.35 (td, J = 3.05, 13 Hz, 1 H), 0.84 (m, 1 H), 0.54 (m, 2 H), 0.12 (m, 2 H); IR ($CHCl_3$) 3379, 3020, 3008, 2928, 1762, 1492, 1446, 1371, 1192, 1168, 1112 cm^{-1}; CIMS $C_{22}H_{26}INO_4$ requires 496 (MH^+), MH^+ found 496; $[\alpha]_D$ = −236° (*c* 1.126, $CHCl_3$). Anal. ($C_{22}H_{26}INO_4$) C, H, N.

3-Acetoxy-6α-iodo-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (11). A mixture of **9** (base) (3.00 g, 5.80 mmol) and tetraethylammonium iodide (2.98 g, 11.6 mmol) in dry acetonitrile (50 mL) was heated and stirred for 4 h at 80 °C under an argon atmosphere when TLC (system B) and mass spectral analysis indicated that the reaction was complete. The solvent was evaporated in vacuo and the residue was dissolved in $CHCl_3$ (100 mL) and washed with 4 × 40 mL of water and evaporated to give **11** (base) as an oil (2.89 g, 97%). Crystallization from 10 mL of warm 2-propanol afforded 2.22 g (77%) of pure **11**: mp 156–157 °C; ^1H NMR ($CDCl_3$) δ 6.85 (d, J = 8.2 Hz, 1 H), 6.63 (d, J = 8.2 Hz, 1 H), 4.79 (dt, J = 13.5, 3.8 Hz, 1 H), 4.65 (d, J = 3.8 Hz, 1 H), 3.10 (m, 1 H), 3.06 (d, J = 18.6 Hz, 1 H), 2.66 (m, 2 H), 2.61 (dd, J = 19, 6.7 Hz, 1 H), 2.19–2.42 (complex m, 3 H), 2.33 (s, 3 H), 2.04 (m, 1 H), 1.45–1.81 (complex m, 4 H), 1.38 (m, 1 H), 0.85 (m, 1 H), 0.55 (m, 2 H), 0.13 (m, 2 H); IR ($CHCl_3$) 3390, 3027, 2928, 2831, 1767, 1494, 1453, 1371, 1227, 1194, 1158, 1115 cm^{-1}; CIMS $C_{22}H_{26}INO_4$ requires 496 (MH^+), MH^+ found 496; $[\alpha]_D$ = −244° (*c* 0.625, $CHCl_3$). Anal. ($C_{22}H_{26}INO_4$) C, H, N.

6β-Iodo-3,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (1, Ioxy). Compound **10** (base) (5.00 g, 10.1 mmol) was dissolved in a mixture of THF (70 mL) and MeOH (70 mL) and the mixture was treated with concentrated aqueous ammonia solution (35 mL) and stirred for 25 min under an argon

atmosphere at ambient temperature when TLC (system A) indicated the reaction to be complete. The solvent was evaporated in vacuo and the residue was dried under high vacuum to afford a quantitative yield of 1 as a white powder. Compound 1·oxalate was crystallized from 150 mL of boiling 2-propanol and dried in vacuo at 80 °C to afford 5.47 g (quantitative) yield of 1·oxalate: mp 177 °C dec; ^{1}H NMR ($CDCl_3$) (base) δ 6.73 (d, J = 8.2 Hz, 1 H), 6.59 (d, J = 8.2 Hz, 1 H), 4.97 (d, J = 8.1 Hz, 1 H), 3.87 (m, 1 H), 3.06 (m, 1 H), 2.84 (d, J = 18.3 Hz, 1 H), 2.51–2.70 (complex m, 3 H), 2.36 (d, J = 6.5 Hz, 2 H), 2.26 (td, J = 12.3, 4.9 Hz, 1 H), 2.10 (m, 2 H), 1.26–1.50 (complex m, 4 H), 0.84 (m, 1 H), 0.53 (m, 2 H), 0.12 (m, 2 H); IR ($CHCl_3$) 3572, 3330, 3005, 2950, 2927, 2833, 1507, 1455, 1146, 1108, 1032, 909 cm^{-1}; CIMS $C_{20}H_{24}INO_3$ requires 454 (MH^+), MH^+ found 454; $[\alpha]_D$ = −149° (c 1.179, MeOH). Anal. ($C_{22}H_{26}INO_7 \cdot C_3H_8O$) C, H, N.

6α-Iodo-3,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan (2, Epiioxy). Compound 11 (base) (1.90 g, 3.84 mmol) in a mixture of 40 mL of MeOH and 20 mL of THF was treated with concentrated aqueous ammonia solution (10 mL) as described above for 1. The reaction was found to be complete within 30 min at ambient temperature (TLC system A). The solvent was evaporated in vacuo and the residue was dried under high vacuum to afford a quantitative yield (1.74 g) of 2 (base). The oxalate salt was crystallized from 50 mL of a mixture of MeOH and 2-propanol (1:1). The MeOH component was distilled off while the volume was kept constant at 50 mL by addition of 2-propanol. The solution was cooled to 25 °C and the crystals were filtered and washed with 2 × 10 mL of cold (0 °C) 2-propanol followed by ether (10 mL) to yield (after drying overnight in vacuo at 80 °C) 2.06 g (99%): mp 211–212 °C dec; ^{1}H NMR ($CDCl_3$) (base) δ 6.75 (d, J = 8.5 Hz, 1 H), 6.55 (d, J = 8.5 Hz, 1 H), 4.83 (dt, J = 13.5, 3.9 Hz, 1 H), 4.64 (d, J = 3.6 Hz, 1 H), 3.04 (m, 2 H), 2.66 (m, 2 H), 2.57 (dd, J = 18, 6.4 Hz, 1 H), 2.14–2.42 (complex m, 3 H), 2.06 (m, 2 H), 1.33–1.78 (complex m, 5 H), 0.85 (m, 1 H), 0.55 (m, 2 H), 0.13 (m, 2 H); IR ($CHCl_3$) 3575, 3371, 3006, 2928, 2832, 1508, 1461, 1386, 1226, 1147, 1113, 1062, 1030 cm^{-1}; $[\alpha]_D$ = −153° (c 0.868, MeOH); CIMS $C_{20}H_{24}INO_3$ requires 454 (MH^+), MH^+ found 454. Anal. ($C_{22}H_{26}INO_7 \cdot C_3H_8O$) C, H, N.

[^{125}I]-3-Acetoxy-6β-iodo-14-hydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan ([^{125}I]-3-*O*-Acetylioxy, [^{125}I]-10). Carrier-free aqueous [^{125}I]sodium iodide (5.0 mCi) was dried by evaporation under a stream of nitrogen and reconstituted with 40 μL of dry acetonitrile. To this solution was added triflate ester 8 (100 μg) dissolved in 10 μL of dry acetonitrile and the reaction mixture was heated to 76 °C for 1.5 h under a nitrogen atmosphere. The reaction mixture was diluted to 5 mL with distilled water and passed through a C_{18}-SepPak (prewetted with acetonitrile and flushed with water) (Waters Associates). The SepPak was washed with 3 × 10 mL of water to remove unreacted [^{125}I]sodium iodide. The unreacted precursor 8 and [^{125}I]ioxy were eluted with 2 × 0.5 mL of acetonitrile containing 0.1% trifluoroacetic acid (TFA). Counting of the product on a γ-counter (Capintec, Model CRC10 radioisotope calibrator) indicated a yield of 5.0 mCi (100% incorporation of carrier-free ^{125}I). The acetonitrile–TFA solvent was removed by careful evaporation under a stream of nitrogen and the residue was redissolved in acetonitrile–0.1% aqueous trifluoroacetic acid (1:3) and injected into a HPLC machine (Waters Associates) fitted with a Perkin–Elmer C_{18} reverse-phase cartridge column (0.4 × 10 cm; 3-μm particle size). Elution with acetonitrile–0.1% aqueous trifluoroacetic acid (1:3) under isocratic conditions at a flow rate of 0.9 mL/min resulted in a clear separation (see Figure 1) of [^{125}I]-10 (measured using both a UV detector set at 214 nM as well as from cpm/fraction) from the precursor 8. The radiolabeled peak displayed the exact retention time and elution profile as unlabeled ioxy 3-*O*-acetyl ester 10. The radiochemical yield of purified [^{125}I]-10 was found to be 5.0 mCi (100%).

The radiolabeled products were stored at −20 °C and used within 1 week of purification. The material was dried down under a gentle stream of nitrogen and redissolved in normal saline prior to in vivo studies.

[^{125}I]-6β-Iodo-3,14-dihydroxy-17-(cyclopropylmethyl)-4,5α-epoxymorphinan ([^{125}I]ioxy, [^{125}I]-1). Cleavage of the 3-*O*-acetyl group of [^{125}I]-3-*O*-acetylioxy ([^{125}I]-10) was performed starting with 315 μCi of [^{125}I]-3-*O*-acetylioxy dissolved in 50 μL of acetonitrile. To this solution at 25 °C was added 50 μL of concentrated aqueous ammonia solution and the reaction mixture was allowed to stand at 25 °C for 20 min. The reaction solvent was removed by careful evaporation under a stream of nitrogen. The residue was dissolved in 50 μL of 0.1% aqueous TFA–acetonitrile and purified by HPLC as described above for [^{125}I]-3-*O*-acetylioxy to give [^{125}I]-1 (279 μCi, 88.5% yield). The HPLC profile of the reaction product indicated that complete cleavage of the 3-*O*-acetyl group had occurred within 20 min after addition of the ammonia solution. The [^{125}I]ioxy eluted with the exact elution profile of unlabeled ioxy. The product was stored at −20 °C and used within 1 week of purification. The material was dried down under a gentle stream of nitrogen and redissolved in normal saline prior to in vivo studies. The results of these biological studies with [^{125}I]-3-*O*-acetylioxy ([^{125}I]-10) and [^{125}I]ioxy ([^{125}I]-1) will be presented elsewhere.

Acknowledgment. We express our sincere appreciation to the Medical Narcotics Division of Mallinckrodt, Inc., St. Louis, MO, for a generous supply of naltrexone. We also offer our appreciation to Wesley White and Noel Whittaker of the Laboratory of Analytical Chemistry for performing ^{1}H-NMR and mass spectral analyses of all intermediates and final compounds described in this paper.

Registry No. 1, 141392-28-1; 1·oxalate, 141392-29-2; [^{125}I]-1, 141392-33-8; 2, 141392-30-5; 3, 16590-41-3; 4, 20410-98-4; 4·(*R*)-mandelate, 141392-24-7; 5, 49625-89-0; 6, 59888-62-9; 6·oxalate, 96917-40-7; 7, 59888-59-4; 8, 94696-55-6; 9, 141392-25-8; 10, 141392-26-9; [^{125}I]-10, 141392-32-7; 11, 141392-27-0.